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The Secretary of Health, Education, and Welfare has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through January 31, 1980.

Treatment of Osteogenic Sarcoma. I. Effect of Adjuvant High-Dose Methotrexate After Amputation[1]

Steven A. Rosenberg,* Bruce A. Chabner, Robert C. Young, Claudia A. Seipp, Arthur S. Levine, Jose Costa, Thomas A. Hanson, Gordon C. Head, and Richard M. Simon[2,3]

SUMMARY

Thirty-nine patients with extremity osteogenic sarcoma and no discernible metastases were treated with amputation and postoperative adjuvant high-dose methotrexate with leucovorin rescue. One half of the patients were also randomized to receive bacillus Calmette-Guérin by a multiple-puncture technique. Results have been analyzed with a minimum followup of 10 months and a median followup of 27 months. Actuarial analysis estimates that 38% of current protocol patients remain continuously free of disease for 24 months compared to only 17.4% of historical control patients ($P = 0.029$; one-sided generalized Kruskal-Wallis test). Bacillus Calmette-Guérin administered by a multiple-puncture technique had no effect on disease-free interval. Minor differences between current protocol and historical control patients with regard to race, age, histologic type, and site and size of primary tumors do not affect the difference in disease-free interval between these two patient groups. However, current patients had somewhat lower grade lesions and consideration of the patients with grade III and IV lesions only, lessens the difference between current and historical control patients ($P = 0.11$; one-sided generalized Kruskal-Wallis test). High-dose methotrexate was administered with virtually no morbidity and no deaths. The small differences observed in this study between protocol patients treated with surgery plus high-dose methotrexate and historical control patients treated with surgery alone point to the need for a prospective randomized study to establish the role of high-dose methotrexate in the adjuvant treatment of patients with osteogenic sarcoma.

[Cancer Treat Rep 63:739–751, 1979]

Surgical excision, usually involving amputation, as a sole treatment for patients with osteogenic sarcoma results in a 5-year survival of about 20%, with a median survival of about 16 months and a median time to metastatic disease of approximately 10 months (1–5). Recently, it has been reported that single drugs, such as high-dose methotrexate (6,7) or Adriamycin (8,9), or multiple-drug combinations (10,11) used in the postoperative surgical adjuvant period were capable of improving disease-free survival in patients with osteogenic sarcoma.

In June 1975, we began a prospective therapy trial in patients undergoing amputation of extremity osteogenic sarcomas in the absence of known metastatic disease. This therapy trial consisted of three main features: (*a*) all patients received high-dose methotrexate with leucovorin rescue; (*b*) one half of the patients were prospectively randomized to receive weekly bacillus Calmette-Guérin (BCG) by a multiple-puncture technique; and (*c*) all patients who developed pulmonary metastases had

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[1] Received Nov 21, 1978; revised Feb 6, 1979; accepted Feb 22, 1979.

[2] Surgery Branch (*S. A. Rosenberg* and *C. A. Seipp*), Clinical Pharmacology Branch (*B. A. Chabner*), Medicine Branch (*R. C. Young*), Pediatric Oncology Branch (*A. S. Levine*), Pathology Branch (*J. Costa* and *T. A. Hanson*), and Biostatistics and Data Management Section (*R. M. Simon*), Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, and the Diagnostic Radiology Department (*G. C. Head*), Clinical Center, National Institutes of Health, Bethesda, Md.

[3] We gratefully acknowledge the help of Drs. Alan R. Baker, Murray F. Brennan, Paul Chretien, Max Cohen, Ernest V. de Moss, Henry Sears, William F. Sindelar, and Paul H. Sugarbaker, Surgery Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute, who cared for most of the patients treated in this protocol and who contributed greatly to the conduct of this study. Donald White played an important role in the data retrieval.

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thoracotomy for resection of all known disease whenever possible, as soon as the pulmonary metastases were observed on chest roentgenogram or lung tomogram.

We report our experience with 39 consecutive patients treated in this protocol with a minimum followup of 10 months and a median followup of 27 months. This paper deals with the effects of high-dose methotrexate with leucovorin rescue and with the trial of BCG immunotherapy. The effect of aggressive resection of pulmonary metastases in those patients who have recurrence is described in the following paper in this issue (12).

METHODS

Patient Eligibility

All patients had histologically proven osteogenic sarcoma. No patients with paraosteal osteogenic sarcoma were treated in this study. Prior to entering the study all patients had complete hematologic and serum chemical analyses, chest roentgenogram, full lung tomograms, metastatic bone survey, and a technetium polyphosphate bone scan. No clinical evidence of metastatic disease was found in any of these patients by any of these studies. Renal function was evaluated by urinalysis, BUN, serum creatinine, and measurement of creatinine clearance. All patients had a presumed curative amputation of the primary lesion. Patients were stratified based on whether the entire involved long bone was amputated or whether the involved long bone was transected. No patient had received any previous chemotherapy, radiation therapy, or immunotherapy.

Treatment

All patients in this study received an amputation of the involved extremity and in all cases the surgical margins were negative for tumor. Thirty-five of our 39 protocol patients had their amputations at the National Institutes of Health, Bethesda, Md, and four were referred to the National Institutes of Health after undergoing amputations elsewhere.

Chemotherapy was begun in the postoperative period as soon as the wound was healed, generally within 2 weeks of surgery. All patients received high-dose methotrexate with leucovorin rescue. In the first 24 patients, the initial methotrexate infusion was given at a dose of 50 mg/kg. The methotrexate dose was escalated by 50 mg/kg every 3 weeks to a maximum dose of 250 mg/kg, and this dose was maintained for 18 months. In the last 15 patients, the initial methotrexate infusion was 100 mg/kg; in 2 weeks this dose was escalated to 250 mg/kg which was maintained every 3 weeks for 18 months. These last 15 patients also received 2 mg of vincristine iv ½ hour prior to the start of methotrexate. All methotrexate was given as a 6-hour infusion in 500 ml of 5% dextrose in water. Two hours after completion of the methotrexate infusion, leucovorin (15 mg/m^2) was given iv. Leucovorin administration was repeated im or iv every 6 hours for a total of eight doses. Forty-eight-hour methotrexate levels were measured in all patients; any patient having a 48-hour level $> 4 \times 10^{-7}$ M received additional leucovorin at doses of 30–100 mg/m^2 for six additional doses.

All patients were vigorously hydrated prior to the iv infusion of methotrexate. Patients received a dose of 3000 ml/m^2/day containing 300 mEq of sodium bicarbonate/m^2/day beginning 12 hours prior to the drug infusion and continuing for 24 hours from the start of methotrexate infusion. Urine pH was checked prior to and during the methotrexate infusion and bicarbonate dose was increased as necessary to maintain the pH > 7. Serum BUN and creatinine were measured in all patients prior to each methotrexate infusion and creatinine clearance was obtained if any rise in BUN or creatinine had occurred since the previous dose.

After obtaining an informed consent, one half of the patients entering this study were randomized to receive BCG by the multiple-puncture technique using a Heaf gun. Fresh-frozen BCG (supplied by Connaught Laboratories, Ontario, Canada) containing 40 mg in each vial was used. One vial of BCG was administered on Days 7 and 14 of each 21-day methotrexate treatment cycle. No BCG was given the week that methotrexate was administered. Four areas of the trunk were treated at a time. A 2 × 4-cm^2 area was cleaned with acetone and allowed to dry. BCG (0.25 ml) was spread over each of these four areas and injected intradermally with 15 strokes of the Heaf gun on each 2 × 4-cm^2 area. Whenever possible, family members were trained to administer the BCG by this technique at home.

Prior to each course of therapy, patients had complete hematologic and serum chemical analyses. A chest roentgenogram was obtained every 3 weeks and a lung tomogram was obtained if any suspicious lesions were seen. A full lung tomogram was performed every 3 months for the first 2 years.

When suspicious lesions were seen on chest roentgenogram, lung tomography and sometimes computerized axial tomography of the lungs were performed. Any patients developing new pulmonary lesions thought to represent metastatic foci had thoracotomy without delay except when lesions too numerous to resect were identified. The results of thoracotomy are presented in the following paper (12).

Pathology

All pathologic material was examined by at least two pathologists, and whenever doubt existed, additional pathologic opinion was obtained. Pathologic material from 23 protocol patients in this study and from 13 historical control patients was reviewed by two additional pathologists (L. V. Ackerman and H. D. Dorfman).

Biopsy material from all patients in the study group was reviewed in conjunction with the radiographs taken at the time the patients were entered in the study. All pathologic material (gross photographs, roentgenograms, and slides) in the historical control and the study groups was reviewed without knowledge of the clinical course of the patient. Each tumor was characterized as to site of origin in the bone (paraosteal, periosteal, or intramedullary), histologic components, and grade. Histologic subclassification was made according to whether the tumor was osteoblastic, chondroblastic, fibroblastic, anaplastic, telangiectatic, or well-differentiated. Mixtures of the above subtypes were recorded by the components when these were present in roughly equal amounts; otherwise the tumors were classified by the predominant component.

Grade was estimated on the basis of cytologic characteristics and mitotic rate—grade I tumors having the best differentiated cytologic characteristics and sparse mitotic figures and grade IV tumors being composed of pleomorphic cells with hyperchromatic nuclei and having a high mitotic rate. The grade of an individual tumor was estimated according to the highest grade seen on the slides examined.

Radiologic Appearance

All available roentgenograms were reviewed by G. C. Head who classified the lesion as to its radiologic appearance and measured the size of the lesion on multiple roentgenographic views.

Historical Control Patients

The charts of all patients submitted to the National Cancer Institute between 1953 and 1974 with a diagnosis of osteogenic sarcoma were reviewed. Twenty-three patients were identified who fulfilled the criteria for entrance in the current protocol. Review of pathologic material confirmed the diagnosis of osteogenic sarcoma in all 23 patients. These patients had received amputation for treatment of osteogenic sarcoma and had received no chemotherapy or radiation therapy in the postoperative period.

Statistical Analysis

Statistical analyses were performed using actuarial techniques. Generalized Kruskal-Wallis tests for unequal censoring were performed to compare treatment groups (13). Tests for trend were performed as described by Mantel (14).

RESULTS

Patient Characteristics

Between June 1975 and August 1977, 52 patients with a diagnosis of osteogenic sarcoma were evaluated by the Surgery Branch, Clinical Oncology Program, Division of Cancer Treatment, National Cancer Institute. Thirteen patients were excluded from the current study: 11 because of the presence of pulmonary metastases, one because of the diagnosis of extraosseous osteogenic sarcoma, and one with osteogenic sarcoma of the pelvis because no locally curative amputation was possible. Four of the 11 patients excluded because of the presence of pulmonary metastases had normal chest roentgenograms but abnormal lung tomograms.

The remaining 39 patients were treated in the current study. One patient was not offered BCG randomization and thus only 38 patients entered the BCG randomization of this study.

The sex, race, and age distribution of protocol and historical control patients are presented in table 1. The location of the primary lesions and the type of surgery performed are presented in table 2. Fifteen of 17 current protocol patients with distal femur lesions had transmedullary above-knee amputations and only two had hip disarticulation. In contrast, 12 of 14 historical control patients with distal femur lesions had hip disarticulation and only two had above-knee amputations.

The pathologic features of the tumors are shown in table 3. Most of the lesions were of the osteoblastic type and most were grades III and IV.

The size of the bone tumors is shown in table 4. The product of perpendicular diameters on the anterior-pos-

TABLE 1.—Patient characteristics: sex, race, and age distribution

	No. of patients	
	Protocol	Historical control
Sex		
Male	26	15
Female	13	8
Race		
Black	13	3
White	26	20
Age in yrs		
6–10	3	1
11–15	11	10
16–20	10	5
21–25	4	1
26–30	3	2
31–40	5	0
41–50	3	2
> 50	0	2
Median	18	16

TABLE 2.—Patient characteristics: location of primary lesions and type of surgery

	No. of patients	
	Protocol	Historical control
Location of primary lesion		
Lower extremity		
Pelvis	1	0
Femur		
Proximal	6	1
Distal	17	14
Tibia-fibula		
Proximal	8	1
Distal	5	1
Upper extremity		
Humerus		
Proximal	2	4
Distal	0	0
Radius-ulna		
Proximal	0	0
Distal	0	2
	39	23
Site of amputation		
Lower extremity		
Below-knee	1	0
Above-knee	27	4
Hip disarticulation	4	13
Hemipelvectomy	5	0
Upper extremity		
Below-elbow	0	0
Above-elbow	0	2
Forequarter	2	4
	39	23

TABLE 3.—Pathologic features

	No. of patients	
	Protocol	Historical control
Histologic type		
Osteoblastic	31[*]	20[†]
Chondroblastic	2	0
Anaplastic	2	2[‡]
Telangiectatic	1	0
Well-differentiated	2	0
Unknown	1[§]	0
Fibroblastic	0	1
Grade		
I	0	0
II	5	1
III	10	2
IV	23	20
Undetermined[§]	1	0

[*] 6 of 31 patients also had chondroblastic elements; 2 of 31 patients also had telangiectatic elements; 1 of 31 patients had "sclerosing" type.

[†] 4 of 20 patients also had chondroblastic elements; 1 of 20 patients also had telangiectatic elements; 1 of 20 patients also had anaplastic elements; 1 of 20 patients had "sclerosing" type.

[‡] 1 of 2 patients also had telangiectatic elements.

[§] Slides were unavailable for review.

terior roentgenogram projection has been shown since lateral films were not available for all patients.

In summary, the 39 current protocol patients are typical of most series of patients with osteogenic sarcoma although the median age (18 years) is somewhat high. These patients are similar to our historical control patients with regard to sex and age distribution and location and histologic type of the primary lesions. The current protocol contains a greater proportion of black patients, a higher incidence of amputations across the involved bone, fewer grade IV lesions and somewhat smaller lesions than the historical control patients.

Results of Therapy

The results of therapy as of June 1, 1978 (median followup, 27 months), are shown in table 5 and figures 1–8. Actuarial analysis estimates that 38% of the current protocol patients remain continuously free of disease for 24 months compared to only 17.4% of the historical control patients (fig 1) ($P = 0.029$; one-sided generalized Kruskal-Wallis test with unequal censoring). A comparison of patients receiving BCG with those not receiving BCG (fig 2) and a comparison of patients receiving the initial methotrexate dose schedule with those receiving the accelerated methotrexate dose schedule with vincristine revealed no difference in disease-free interval.

Because one third (13 of 39 patients) of all current protocol patients were black compared to only three of 23 historical control patients (13%), we examined the ef-

TABLE 4.—Size of tumor on roentgenogram

Tumor size[*] (cm^2)	No. of patients (%)	
	Protocol	Historical control
0–20	2 (5.1)	2 (8.7)
21–40	7 (17.9)	1 (4.3)
41–60	9 (23.1)	0 (0)
61–80	5 (12.8)	2 (8.7)
81–100	3 (7.7)	4 (17.4)
101–120	3 (7.7)	4 (17.4)
121–140	1 (2.6)	1 (4.3)
141–160	1 (2.6)	2 (8.7)
161–180	0 (0)	1 (4.3)
181–200	1 (2.6)	0 (0)
> 200	0 (0)	2 (8.7)
Undetermined	7 (17.9)	4 (17.4)
Totals	39	23
Mean product of diameters	65.9	113.0

[*] Product of perpendicular diameters on anterior-posterior roentgenogram.

Table 5.—Results of treatment*

	No. of—			
	Patients	Recurrences	Median (mos)	
All protocol patients	39	22 (56.4%)	10.7	$P = 0.029$
Historical control patients	23	19 (82.6%)	6.0	
Plus BCG	18	10	11.6	$P < 0.2$
No BCG	20	11	8.9	
Initial dose schedule	24	14	10.0	$P < 0.5$
Accelerated dose schedule	15	8	10.4	

*All *P* values were determined by a 1-sided generalized Kruskal-Wallis test with unequal censoring.

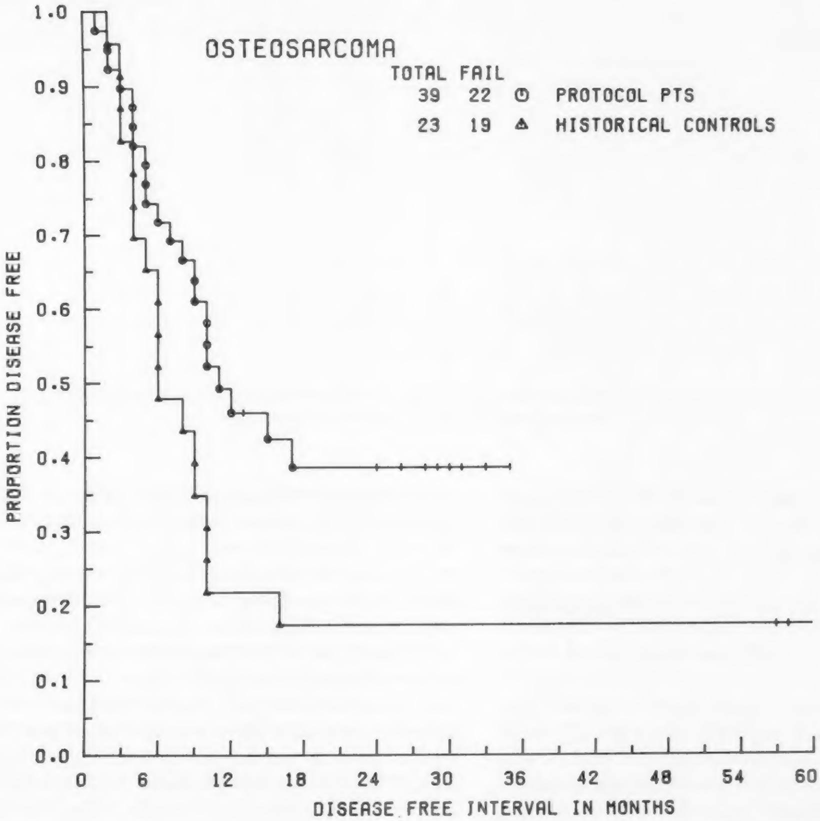

Figure 1.—Actuarial analysis of disease-free interval in protocol patients compared to historical control patients. Disease-free interval is improved in protocol patients ($P = 0.029$; 1-sided generalized Kruskal-Wallis test).

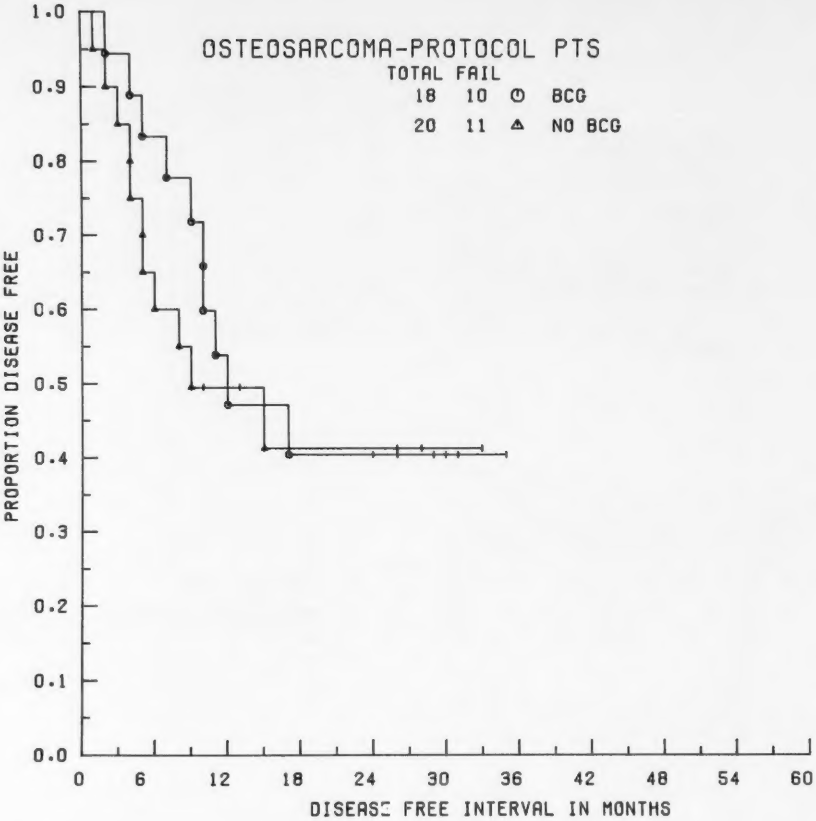

FIGURE 2.—Actuarial analysis of disease-free interval in patients treated with BCG compared to those not receiving BCG. There is no statistical difference between these groups.

fect of race on disease-free interval. Although the black patients did somewhat better, this difference does not approach statistical significance ($P = 0.25$; two-sided Kruskal-Wallis test). However, the difference in disease-free interval between protocol and historical control patients is decreased when the comparison is limited to white patients (fig 3; $P = 0.14$; one-sided Kruskal-Wallis test).

Because primary tumors in the current protocol patients tended to be smaller than those in the historical control patients, we examined the effect of size on disease-free interval. Protocol patients with larger tumors (> 60 cm^2; product of perpendicular diameters) did somewhat better than those with smaller tumors, although this was not statistically significant ($P = 0.22$). Consequently, comparison of only those patients with tumors > 60 cm^2 results in an even larger difference between protocol and historical control patients (fig 4; $P = 0.023$; one-sided Kruskal-Wallis test).

Increasing age did appear to correlate with improved prognosis in the current protocol group (fig 5; $P = 0.07$; two-sided Mantel-Haenszel test for trend), although the protocol and historical control patients were quite comparable and this factor did not affect the analysis of methotrexate effect.

Similarly, the site of the primary tumor did not correlate with prognosis in our current protocol patients. Thus, comparison of only those patients with femur tumors also demonstrated an improved prognosis of current protocol patients compared to historical control patients (fig 6; $P = 0.06$; one-sided Kruskal-Wallis test). This was important to analyze because only 67% of the protocol patients had tumors above the elbow and knee compared to 87% of the historical control patients.

Most of the tumors in both the protocol and historical control patients were of the osteoblastic type (79% and 87% respectively). Comparison of disease-free intervals between only those protocol and historical control pa-

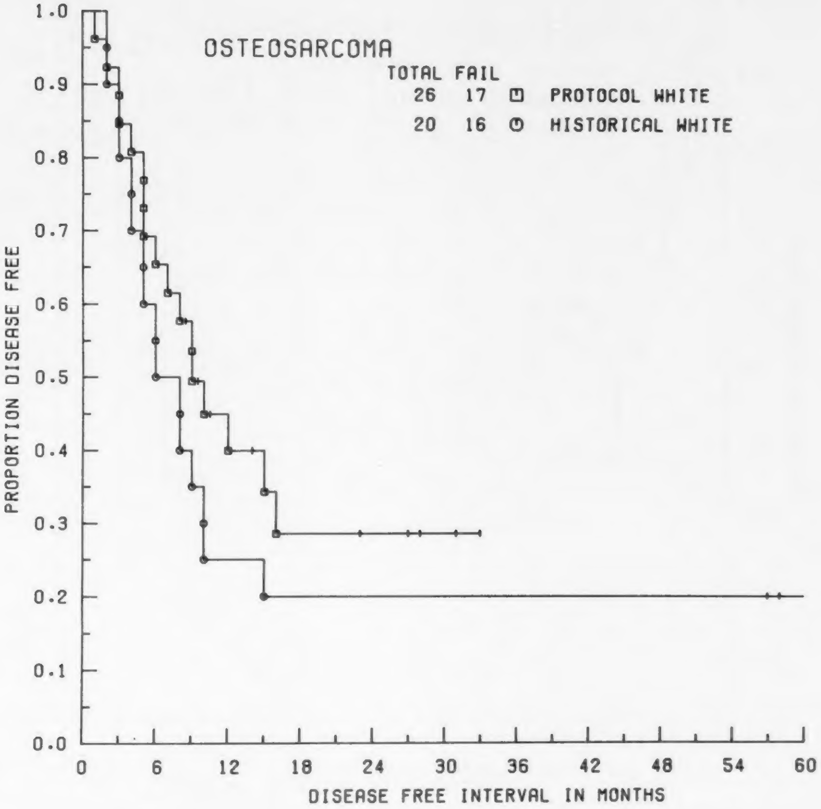

FIGURE 3.—Disease-free interval in white protocol patients compared to white historical control patients (P = 0.14; 1-sided Kruskal-Wallis test).

tients with osteoblastic lesions revealed an improvement in current patients ($P = 0.06$; one-sided Kruskal-Wallis test).

Increasing histologic grade did appear to correlate with poorer prognosis in the current protocol group (fig 7; $P = 0.034$; one-sided Mantel-Haenszel test for trend). However, the differentiation of grade III from grade IV lesions is highly subjective. When one of our pathologists re-reviewed ten grade III and ten grade IV lesions from the current protocol without a knowledge of the previous grading, three previous grade III lesions were categorized as grade IV and three previous grade IV lesions were categorized as grade III. When grade III and IV lesions are combined and analyzed versus grade III and IV lesions from historical control patients, the benefit seen in current protocol patients is decreased (fig 8; $P = 0.11$; one-sided Kruskal-Wallis test).

It should be noted that the actuarial recurrence curves of all current protocol and historical control patients are virtually identical for the first 6 months following amputation, although after this point recurrences tend to be less frequent in the methotrexate-treated group.

Chemotherapy

Four hundred and seventy-six doses of high-dose methotrexate with leucovorin rescue have been administered to our 39 protocol patients as of June 1, 1978. Twenty-three of the 39 patients received > 90% of their methotrexate doses at maximum escalation and with no delays. Four patients had delays of at least 1 week for one to three courses of methotrexate. Escalation to maximum dose (250 mg/kg) was not achieved in six patients because of problems with renal or hepatic dysfunction, although all of these patients received at least 100 mg/kg as their regular maximum dose. Four patients discontinued methotrexate therapy after receiving six, seven, 14, and 20 doses respectively. Two patients developed an anaphy-

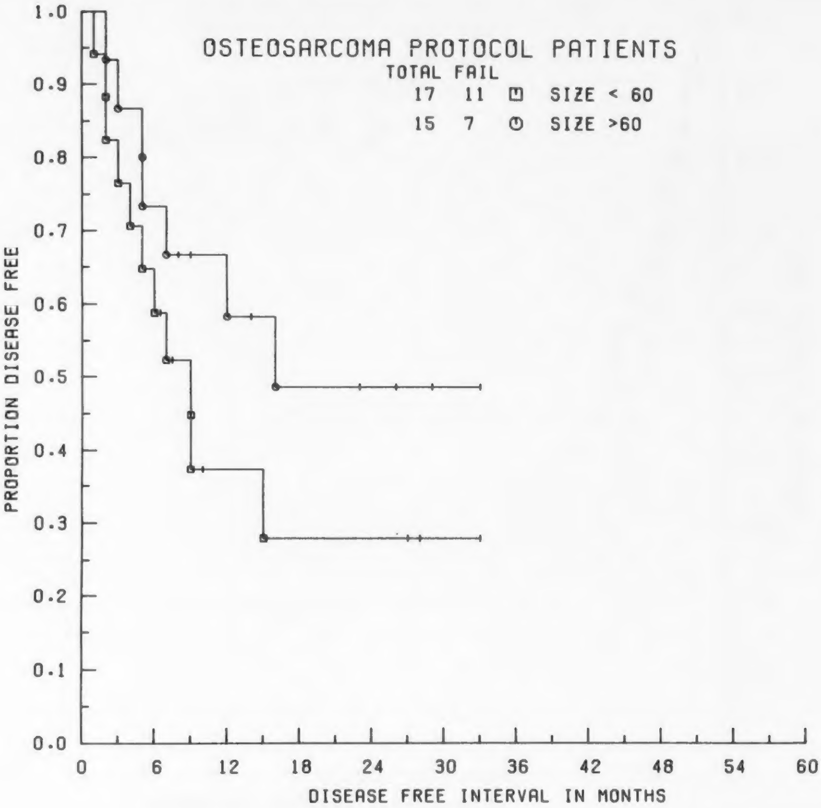

FIGURE 4.—Actuarial analysis of disease-free interval in current protocol patients related to the size of the primary lesion. Protocol patients with larger tumors did somewhat better than those with smaller tumors, although this was not statistically significant ($P = 0.22$; 1-sided Kruskal-Wallis test).

lactic reaction to methotrexate after receiving seven and nine doses respectively that required discontinuance of methotrexate therapy; these patients have been previously reported (15).

It should be pointed out that the vigorous hydration used in our patients did not affect methotrexate blood levels or clearance rates compared to lesser hydration (16).

There was no difference in recurrence rate related to these chemotherapy administration irregularities. Fifteen of the 23 patients receiving methotrexate administration exactly as stipulated by the protocol had recurrence and eight did not. Two patients with delayed doses had recurrence and two did not. Three patients with less than maximum escalation had recurrence and three did not. The patient who discontinued therapy after seven doses had recurrence, although the other three patients who discontinued therapy did not.

With the exception of the two anaphylactic reactions to methotrexate therapy, one of which was associated with severe drug toxicity (15), there were no severe methotrexate complications and all patients were effectively rescued with no significant bone marrow suppression.

Bacillus Calmette-Guérin Immunotherapy

Four hundred and fifteen doses of BCG were administered by scarification to the 16 randomized patients. Two additional patients who were randomized to receive BCG subsequently refused it. Of the 16 patients receiving BCG, family members performed the scarification in ten patients and it was applied by a physician in six. Thirteen of the 16 patients received > 90% of their doses on time and in the remaining three patients either severe ulcer-

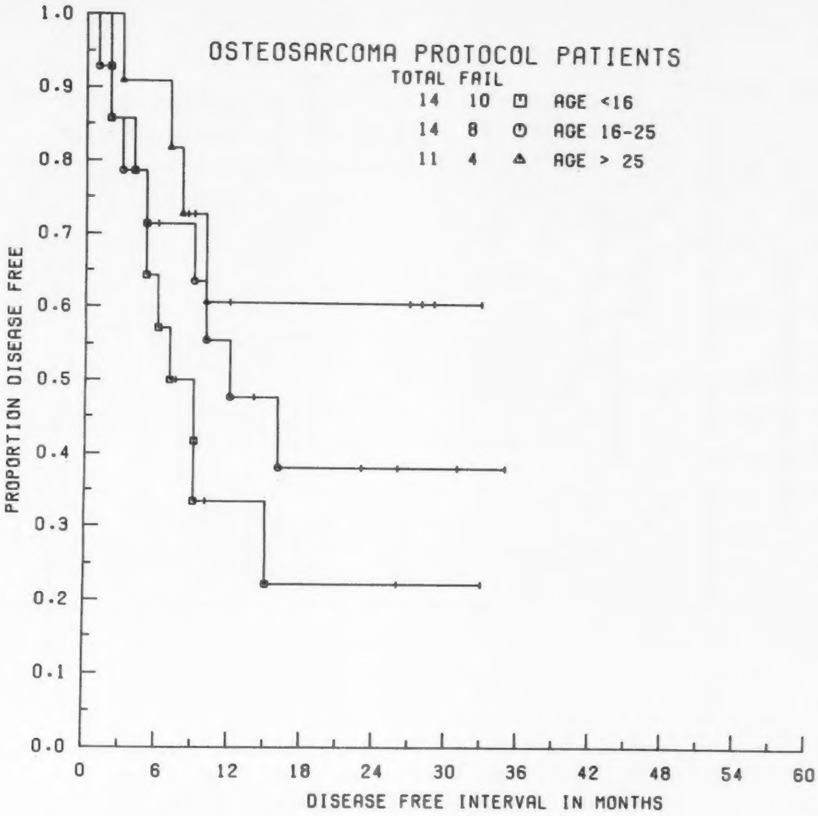

FIGURE 5.—Actuarial analysis of disease-free interval in current protocol patients related to age. Increasing age did appear to correlate with improved prognosis in the current protocol group (P = 0.07; 2-sided Mantel-Haenszel test for trend).

ation or patient refusal led to discontinuance of the BCG after a minimum of 14 doses.

All patients experienced some ulceration at the BCG administration site which became worse after multiple treatments. After ten to 15 treatments, all patients required dilution of the BCG to approximately one fifth of the starting concentration to make the local inflammation and ulceration side effects tolerable. With the exception of occasional mild malaise and low-grade fever and the scarring resulting from scarification, no other side effects due to the BCG administration were seen.

DISCUSSION

In 1974, Jaffe et al (6) reported that high-dose methotrexate with leucovorin rescue administered in the immediate postamputation period to 12 patients with osteogenic sarcoma resulted in prolonged disease-free survival compared to historical control patients. Other groups using either single-agent or combination-agent adjuvant chemotherapy have also reported improvement in disease-free survival greater than the 15%–20% 2-year disease-free survival rate expected for patients treated with surgery alone (8–11).

In June 1975, we began a prospective trial in patients with extremity osteogenic sarcoma. Three main therapeutic maneuvers were examined:

1. Treatment of all patients with high-dose methotrexate and leucovorin.
2. Randomization of one half of the patients to receive BCG by a multiple-puncture technique.
3. Careful postoperative screening and aggressive resection of pulmonary metastatic disease when it occurred.

This paper presents our results with the treatment of 39 consecutive patients seen between June 1975 and August 1977 with resectable osteogenic sarcoma in the absence of clinical evidence of pulmonary metastases. These

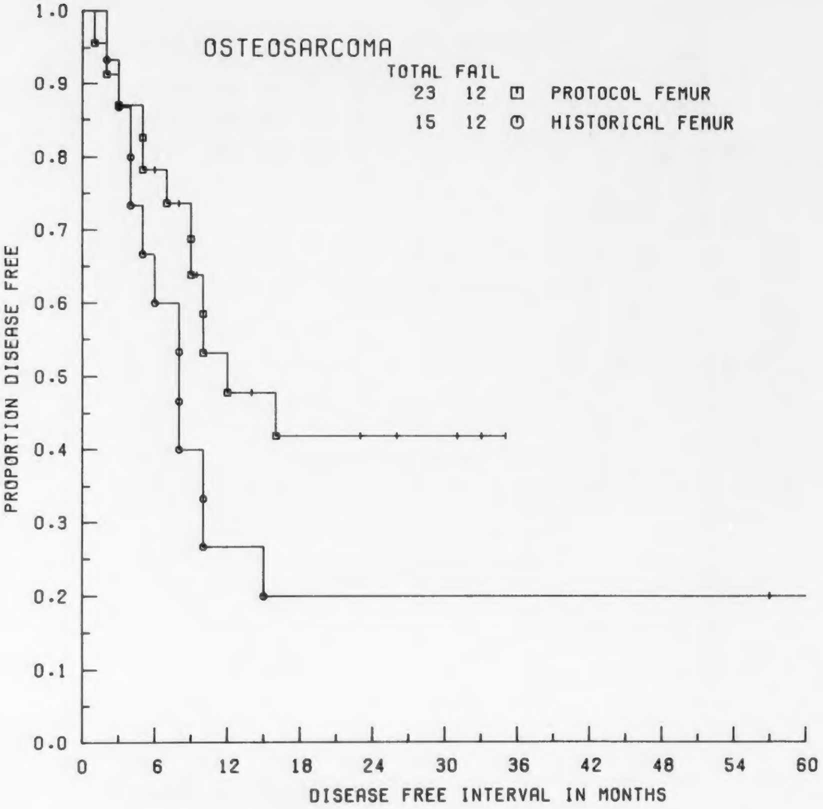

FIGURE 6.—Actuarial analysis of disease-free interval in protocol patients with femur lesions compared to historical control patients with femur lesions ($P = 0.06$; 1-sided Kruskal-Wallis test).

patients have been compared to our own series of 23 patients with osteogenic sarcoma treated by surgery alone prior to 1974. The disease-free and overall survival curves of our historical control patients (17.4% and 21.7% respectively) are virtually identical to those of many reported series of patients treated with surgery alone (1–5). For example, reported 5-year disease-free survival rates using surgery alone were 20% (Mayo Clinic, Rochester, Minn) (3); < 20% (Boston Children's Hospital, Mass) (5); 18% (Memorial Sloan-Kettering Cancer Center, New York, NY) (17); and 17% (M. D. Anderson Hospital, Houston, Tex) (4). Analysis of disease-free survival rates with surgery alone showed no significant improvement with time over the last 15 years with the exception of a recent report from the Mayo Clinic indicating an improvement in disease-free survival in patients treated with surgery alone between 1972 and 1974 (18).

Seventeen (43.6%) of our current 39 patients have remained continuously free of disease and the actuarial estimate of the 2-year continuous disease-free survival rate is 38%. The median time to recurrence in our protocol patients was 10.7 months compared to 6.0 months in our historical control patients ($P = 0.029$; one-sided generalized Kruskal-Wallis test with unequal censoring). Since followup on some of our patients is only 10 months, there may be additional recurrences. Five (41.7%) of the 12 patients treated with high-dose methotrexate and leucovorin originally reported by Jaffe et al in 1974 (6) have remained disease-free with one patient having recurrence 4 years after amputation (7).

Role of High-Dose Methotrexate

This modest improvement in disease-free survival based on a comparison with historical control patients, if attributable to high-dose methotrexate, is of importance in the treatment of patients with osteogenic sarcoma. Was this improvement due to the use of high-dose

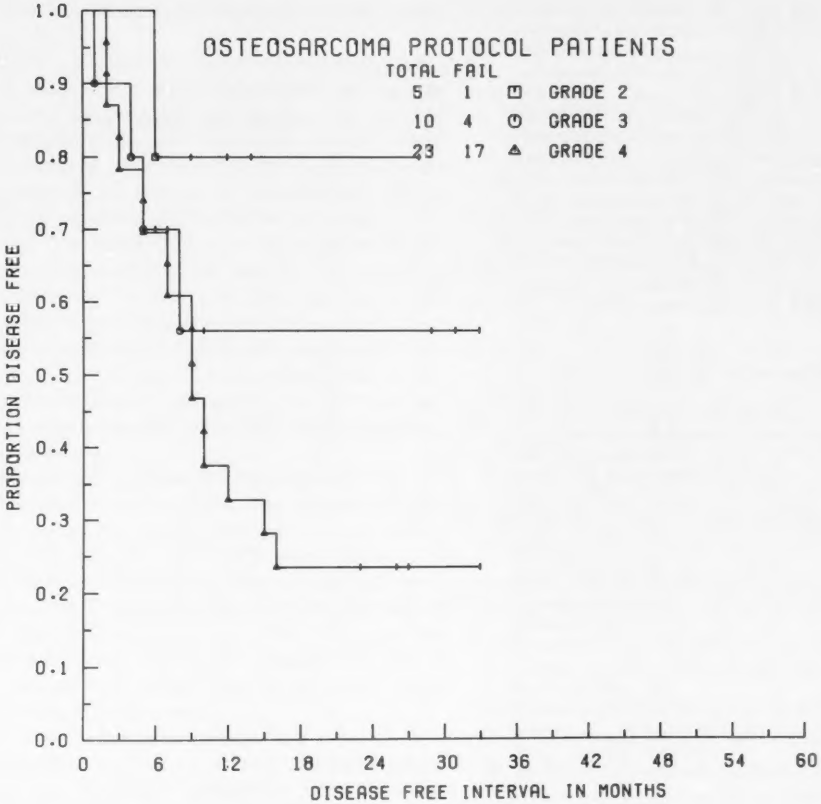

FIGURE 7.—Actuarial analysis of disease-free interval in current protocol patients as a function of the histologic grade of the primary lesion. Increasing histologic grade correlated with poor prognosis ($P = 0.034$; 1-sided Mantel-Haenszel test for trend).

methotrexate? Several alternate explanations must be considered. Our current protocol patients appear to be similar to our historical control patients who are in turn similar to the historical control patients reported by many others. Minor differences between current protocol and historical control patients with regard to race, age, histologic type, and site and size of primary tumors do not affect the difference in disease-free interval between these two patient groups. Histologic grade, however, is an important prognostic indicator and our current protocol patients have a slightly lower incidence of grade III and IV lesions (33 of 39 patients) than do our historical control patients (22 of 23 patients). When comparing only those patients with grade III and IV lesions there is a decrease in the difference in disease-free interval between current and historical control patients (fig 8; $P = 0.11$).

All current protocol patients received both preoperative chest roentgenograms and lung tomograms. Eleven of 52 patients referred to us with a diagnosis of osteogenic sarcoma were found to have pulmonary metastases and were thus ineligible for the study. Four of these 11 patients had normal chest roentgenograms and were diagnosed as having pulmonary metastatic disease only on chest tomography. This is similar to the experience at the Sidney Farber Cancer Institute, Boston, Mass, where Frei et al (7) have reported one of a total of 53 patients with osteogenic sarcoma presenting with a normal chest roentgenogram but abnormal tomograms. Thus, it appears that additional screening with lung tomograms may eliminate small numbers of patients with pulmonary metastases from current adjuvant studies. This effect is likely to be small, however, and in 13 of 20 (in three patients this information could not be recovered) of our own historical control patients, preoperative tomograms had also been performed. If the four current protocol patients excluded because of positive tomograms are included in the analysis with a hypothetic recurrence

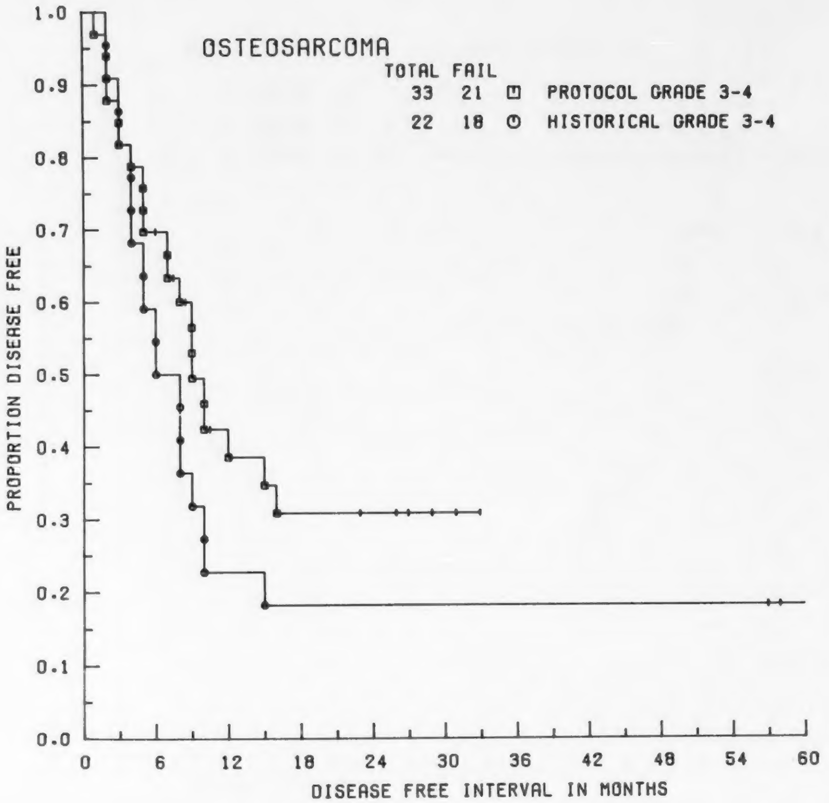

FIGURE 8.—Actuarial analysis of disease-free interval in current protocol patients with grade III and IV lesions compared to historical control patients with grade III and IV lesions ($P = 0.11$; 1-sided Kruskal-Wallis test).

at 3 months, then the difference between the current protocol and historical control patients is somewhat reduced ($P = 0.11$; one-sided Kruskal-Wallis test).

However, current protocol patients did receive postoperative chest roentgenograms every 3 weeks and lung tomography every 3 months, thus increasing the chance that pulmonary metastases would be detected earlier than in our historical control patients. This careful screening for the appearance of pulmonary metastases would tend to minimize real differences in comparison of disease-free intervals with historical control patients and increase our confidence that our observed difference is a real one.

An important factor in comparing current adjuvant trials with historical control patients is the time from surgery to entrance in the treatment protocol. This is especially important in patients with osteogenic sarcoma since > 50% of recurrences occur during the first 12 months in patients treated with surgery alone. In our current protocol, 33 of 39 patients received their first chemotherapy dose within 30 days after amputation and an additional two patients began chemotherapy between 30 and 40 days after amputation. The remaining four patients began chemotherapy at 80, 88, 98, and 119 days after amputation, usually because of problems with wound healing. Of these latter four patients, two have had recurrence. Deletion of these latter four patients does not change any of the conclusions of this study.

Information concerning time from operation to entrance in a treatment evaluation protocol is difficult to obtain in many published studies. In our series early recurrences were common and three patients had recurrence after just a single dose of methotrexate. Twelve of our 22 patients who had recurrence did so during the first 6 months. This is in marked contrast to results with the use of high-dose methotrexate at the Sidney Farber Cancer Institute where no patients had recurrence during the first 6 months (7), and may be due to differing intervals from time of operation to entrance in the treatment protocols.

In summary, a modest but statistically significant prolongation of disease-free survival has been seen in our current protocol patients and may be due to the use of high-dose methotrexate with leucovorin rescue; other differences between the current protocol and historical control patients do not account for the observed differences, with the possible exception of the histologic grade of the lesion. The exact influence of this latter factor cannot be determined from this analysis. In examining a variety of patients, it is always possible that some factor will be found by chance alone that provides an alternative explanation to an observed significant overall difference in disease-free survival that is truely attributable to an improvement in treatment. We cannot rule out this possibility, particularly because of the difficulties in grading lesions histologically.

In the absence of a prospective randomized study, it is impossible to be certain that the use of high-dose methotrexate has improved the disease-free interval in current protocol patients. The small differences observed in this study between protocol patients treated with surgery plus high-dose methotrexate and historical control patients treated with surgery alone point to the need for a prospective randomized study to establish the role of high-dose methotrexate in the adjuvant treatment of patients with osteogenic sarcoma.

This paper shows the effect of high-dose methotrexate on the disease-free interval in patients with primary osteogenic sarcoma. Patients who had recurrence were vigorously treated with thoracotomy to resect pulmonary disease, and our overall treatment strategy has resulted in a dramatic improvement in overall survival ($P < 0.001$). This aspect of our treatment regimen is presented in the following paper in this issue.

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Treatment of Osteogenic Sarcoma. II. Aggressive Resection of Pulmonary Metastases[1]

Steven A. Rosenberg,* M. Wayne Flye, David Conkle, Claudia A. Seipp, Arthur S. Levine, and Richard M. Simon[2]

SUMMARY

Thirty-nine patients with osteogenic sarcoma were treated in a prospective protocol involving the use of adjuvant high-dose methotrexate, frequent screening for pulmonary metastases, and aggressive resection of all metastatic disease whenever possible. Twenty-two of these 39 patients have had recurrence and in 20 patients evidence of metastatic disease was confined to the lungs. Eighteen of the patients had thoracotomy, and in 11 patients all known disease was resected. Although four of these patients have required further thoracotomies, all 11 patients have no evidence of disease. Thus, of the original 39 patients, 30 (76.9%) are now alive and 28 (71.8%) have no evidence of disease, with a median followup of 27 months. Survival is significantly improved compared to historical control patients ($P < 0.001$; one-sided Kruskal-Wallis test).

[Cancer Treat Rep 63:753–756, 1979]

In the preceding paper in this issue, we described our prospective trial of therapy for patients undergoing amputation of extremity osteogenic sarcomas in the absence of known metastatic disease (1). All patients received adjuvant high-dose methotrexate with leucovorin rescue and one half of the patients were prospectively randomized to receive bacillus Calmette-Guérin. Twenty-two (56.4%) of 39 consecutive patients in this protocol have had recurrence, with a minimum followup of 10 months and a median followup of 27 months. These patients had early aggressive resection of all metastatic disease whenever possible. This paper describes our results with this approach. Of the original 39 patients, 30 (76.9%) are now alive and 28 (71.8%) have no evidence of disease. Survival is significantly improved in the current protocol group compared to the historical control group ($P < 0.001$; one-sided generalized Kruskal-Wallis test).

METHODS

Patient eligibility criteria and stratifications were described in detail in the preceding paper (1). In brief, all patients with extremity osteogenic sarcoma had amputation and received high-dose methotrexate with leucovorin rescue for 18 months postoperatively or until recurrence. Methotrexate was rapidly escalated to 250 mg/kg and was maintained at this dose. The last 15 patients in the protocol also received vincristine (2 mg/m^2) prior to the methotrexate.

Prior to entrance in the protocol all patients had normal chest roentgenograms and full-lung tomograms. After surgery, chest roentgenograms were obtained every 3 weeks and lung tomography was performed every 3 months for the first 2 years.

When suspicious lesions were seen on chest roentgenograms, lung tomography and sometimes computerized axial tomography of the lungs were performed. Any patients developing new pulmonary lesions thought to represent metastatic foci had early thoracotomy except when lesions too numerous to resect were identified. Thoracotomy was generally performed via a lateral approach, although in some patients with bilateral metastases a median sternotomy with simultaneous resection of metastatic foci in both lungs was performed. Small wedges

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of the lung containing suspicious nodules were excised using an automatic staple technique.

Followup chest roentgenograms and tomograms on patients in the historical control group were more sporadic and varied widely from patient to patient.

Statistical Analysis

Statistical analyses of the current protocol patients and 23 comparable historical control patients were performed using actuarial techniques. Generalized Kruskal-Wallis tests for unequal censoring were performed to compare treatment groups (2).

RESULTS

Twenty-two (56.4%) of 39 current protocol patients and 19 of 23 historical control patients had recurrence, with a median disease-free interval of 10.7 months and 6.0 months respectively ($P = 0.029$; one-sided Kruskal-Wallis test). The pattern of recurrence is presented in table 1. Twenty of the 22 current protocol patients had pulmonary disease as the first sign of recurrence. One patient who had an extended hemipelvectomy for a pelvic tumor developed a local recurrence and one patient had recurrence in a previous bone chip donor site. With the exception of one patient who had local recurrence, all historical control patients had recurrence in the lungs first.

In all but two of the current patients, the first evidence of pulmonary recurrence was seen on the plain chest roentgenogram. In two patients, routine tomograms provided the first evidence of recurrence at a time when the plain chest roentgenogram was normal.

The pattern of pulmonary recurrence is presented in table 2. In 12 of 20 protocol patients, a single unilateral lesion was first observed clinically on radiologic examination, but only nine of these patients had a single unilateral lesion at thoracotomy. In contrast, only three of 18 historical control patients had single unilateral lesions clinically at the time of initial discovery and all three had a solitary lesion at thoracotomy.

TABLE 1.—Recurrence in osteogenic sarcoma patients

	No. of patients	
	Protocol	Historical control
No. of patients	39	23
No. of patients with recurrence	22 (56.4%)	19 (82.6%)
Site of first recurrence		
Lungs	20	18
Local	1*	1†
Bone chip donor site	1	—

*In the pelvis of a patient who had extended hemipelvectomy for pelvic tumor.

†In the hip of a patient who had hip disarticulation for femur tumor.

TABLE 2.—Pattern of pulmonary recurrence

	No. of patients	
	Protocol	Historical control
No. of patients with pulmonary recurrence	20	18
Radiologic findings		
Unilateral, single	12	3
Unilateral, multiple	1	1
Bilateral	7	12
Unknown	0	2
No. of patients undergoing thoracotomy	18	6
Findings at thoracotomy		
Unilateral, single	9	3
Unilateral, multiple	4	0
Bilateral	5	3
No. of patients with no evidence of disease after thoracotomy	11*	3

*All of these patients had no evidence of disease as of June 1978.

Eighteen of 20 protocol patients had thoracotomy compared to six of 18 historical control patients. Eleven of 18 protocol patients undergoing thoracotomy had resection of all known metastatic disease and in seven patients the disease was too extensive to resect. All of these latter seven patients were treated with vigorous combination chemotherapy. Six have died and one is presently alive with extensive disease at 194 days after thoracotomy.

Treatment of the 11 patients with no evidence of disease after pulmonary resection varied considerably. Two patients received no chemotherapy after thoracotomy and one patient subsequently had recurrence. Four patients received Adriamycin (50 mg/m^2) and cyclophosphamide (500 mg/m^2) every 4 weeks with one recurrence, and five received a combination regimen of vincristine, high-dose methotrexate with leucovorin, cyclophosphamide, Adriamycin, DTIC, melphalan, and *cis*-dichlorodiammineplatinum(II) with two recurrences. One patient in this latter treatment group died as a result of the complications of chemotherapy and had no evidence of disease at autopsy. The number of patients receiving each of these additional chemotherapy treatments after recurrence is too small to assess the effectiveness of the chemotherapy.

Thus, four of 11 patients with resected pulmonary recurrences had a second recurrence, all with further pulmonary disease. All four patients had one or two additional thoracotomies and were once again rendered free of disease; they remain disease-free as of June 1978. In eight of these 11 patients > 12 months has passed from the time of the first recurrence and in four > 24 months has passed. To summarize (fig 1), of the 39 current protocol

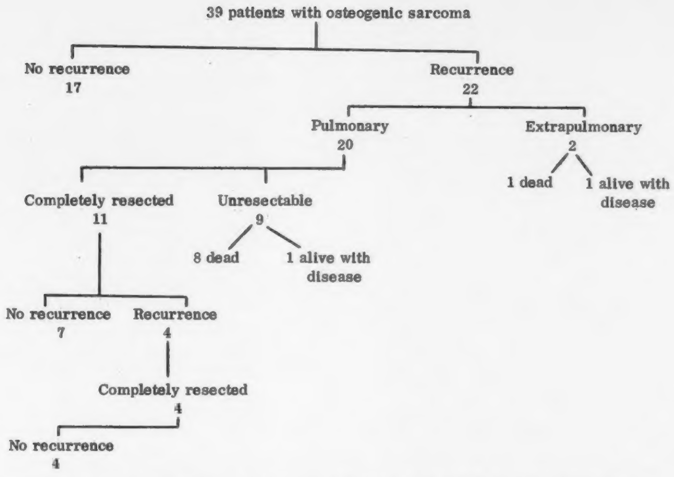

FIGURE 1.—Summarization of 39 protocol patients receiving adjuvant chemotherapy.

patients receiving adjuvant chemotherapy, 17 have never had recurrence and an additional 11 have no evidence of disease after one or more thoracotomies. Improvement in the overall survival of current protocol patients with osteogenic sarcoma compared to that of historical control patients is highly significant (fig 2; $P < 0.001$; one-sided Kruskal-Wallis test). Twenty-eight (71.8%) of 39 patients currently have no evidence of disease, with a median followup of 27 months.

DISCUSSION

Our treatment strategy for patients with osteogenic sarcoma included the use of adjuvant high-dose methotrexate with leucovorin rescue. One half of the patients were prospectively randomized to receive bacillus Calmette-Guérin by a multiple-puncture technique. The results of this adjuvant trial were described in detail in the preceding paper (1).

Many investigators have advocated the aggressive resection of metastatic pulmonary nodules in patients with osteogenic and soft tissue sarcomas (3–10). In most series resection of *all* metastatic disease leads to long-term survival in 20%–30% of patients. Thus, we also incorporated very frequent screening for the detection of pulmonary metastases and aggressive resection of these metastases in our treatment regimen for patients with osteogenic sarcoma.

In an attempt to detect pulmonary metastatic disease as early as possible, all patients received chest roentgenograms every 3 weeks after amputation. It is of interest that 12 of 20 current protocol patients with pulmonary recurrence had only a single unilateral lesion at the time of detection compared to only three of 18 historical control patients. This was further reflected in the findings at thoracotomy where nine protocol patients but only three historical control patients had single metastases. Two possible reasons for this difference should be considered. Current protocol patients received high-dose methotrexate and this chemotherapy may have decreased the frequency of pulmonary metastases. A more likely explanation, however, is that the more frequent chest roentgenograms of the current group led to earlier detection of metastases. Because screening for pulmonary metastatic disease was sporadic in the historical control patients, it is not possible to distinguish the relative contribution of these two factors.

It is noteworthy that all 11 patients who had recurrence and had resection of all known pulmonary disease are alive and free of disease as of June 1978. Four of these patients had recurrence after the first thoracotomy but could again be made disease-free by repeat thoracotomy. This is in marked contrast to the nine patients with unresectable pulmonary disease (seven had attempted thoracotomy). Eight of these nine patients are dead and one is alive with extensive disease.

The effectiveness of aggressive chemotherapy following complete resection of pulmonary metastases in patients with osteogenic sarcoma cannot be decided by the results of this study since it is not possible to distinguish the effects of chemotherapy from the effects of aggressive screening and early resection of pulmonary metastases.

Using the treatment strategy outlined in this paper, 28 (71.8%) of 39 patients presenting with osteogenic sarcoma were free of disease, with a median followup of 27 months. This represents a significant improvement in survival compared to that of the historical control patients ($P < 0.001$).

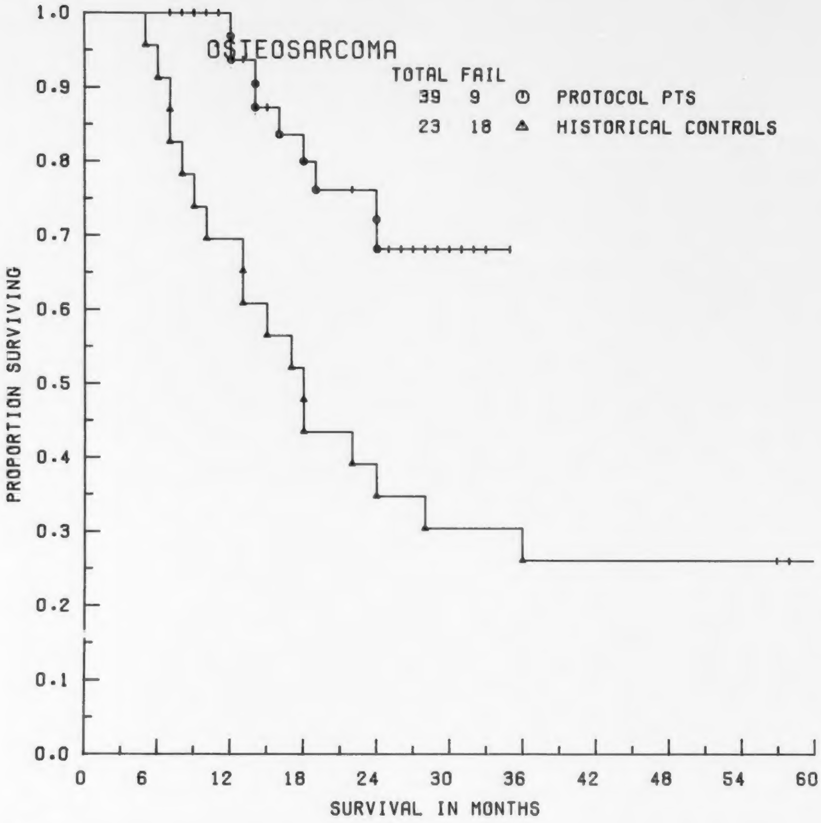

FIGURE 2.—Actuarial analysis of survival in current protocol and historical control patients ($P < 0.001$; 1-sided Kruskal-Wallis test).

As is the case in many current treatment trials, multiple changes in therapy occurred simultaneously and it is difficult to distinguish among the effects of any single treatment maneuver. In this and the preceding paper (1) we have attempted to analyze separately the effects of adjuvant high-dose methotrexate and the effects of aggressive screening and thoracotomy for pulmonary metastases. It is clear, however, that an overall dramatic improvement in disease-free survival has resulted from the application of new treatment and supportive strategies for patients with osteogenic sarcoma. Prospective randomized trials will be required to determine the exact role of each component in these strategies.

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High-Dose Methotrexate for Advanced Breast Cancer[1,2]

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SUMMARY

High-dose infusions of methotrexate with citrovorum factor rescue were evaluated in 27 patients with advanced recurrent breast cancer who had previously been treated with various Adriamycin-containing regimens. Eight of 27 patients (29%) achieved objective tumor regression with a median duration of response of 26 weeks. Nineteen patients had previously received standard doses of methotrexate (< 50 mg/m²/dose), while eight patients had had no prior exposure to methotrexate. The response rates observed in these two groups of patients were similar. Except for two drug-related deaths, toxic effects were acceptable. Myelosuppression was mild, transient, and noncumulative. Gastrointestinal toxic effects did not appear to be dose-related and were mild in most instances. Central nervous system dysfunction with lethargy, fatigability, confusion, and disorientation was the most significant toxic effect of this high-dose methotrexate therapy and was observed in six (22%) of the patients. In two patients treatment with this program was discontinued because of the development of renal dysfunction. High-dose methotrexate with citrovorum factor rescue appears to be an effective regimen in patients with advanced refractory breast cancer. However, in view of the enormous cost necessitated by this treatment approach, we do not feel further studies would be worthwhile.

[Cancer Treat Rep 63:757–761, 1979]

Methotrexate is a well-established agent in the treatment of patients with advanced breast cancer. It has been studied extensively as a single agent or, more recently, in various combinations with cyclophosphamide, 5-fluorouracil (5-FU), vincristine, and prednisone (1–6). Used as a single agent in conventional doses (< 50 mg/m²/dose), it has produced a response rate of 20%–35% (7–11). In recent years, several investigators have reported on the use of high-dose methotrexate (> 50 mg/m²/dose) with citrovorum factor rescue in patients with various solid tumors (12–16). Based on their encouraging results and the relative safety of this program, we initiated a pilot study in patients with refractory breast cancer; this paper reports our results with the first 39 patients entered.

MATERIALS AND METHODS

Thirty-nine patients with biopsy-proven breast cancer and evidence of progressive metastatic disease after various Adriamycin-containing regimens were entered in this study. The criteria for patient eligibility included absence of renal dysfunction, defined as a serum creatinine of > 1.5 mg/dl or a creatinine clearance of < 60 ml/minute, adequate bone marrow function, defined as a peripheral absolute granulocyte count of > 1500/mm³ and a platelet count of > 100,000/mm³, and adequate liver function, defined as a total bilirubin of < 2 mg/dl, unless the inadequacy was due to progressive tumor. Patients with a large third-space reservoir, that is, ascites or pleural effusion, were excluded. Included in the study were patients with demonstrable refractoriness to pre-

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[3] Medical Breast Service, Department of Medicine (*H.-Y. Yap, G. R. Blumenschein, G. N. Hortobagyi*, and *C. K. Tashima*), and Departments of Developmental Therapeutics (*B.-S. Yap, R. S. Benjamin*, and *G. P. Bodey*) and Pediatrics (*A.-Y. Wang*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

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vious therapy with conventional doses of methotrexate. Informed consent was obtained from all patients.

The starting dose of methotrexate was 2.5 g/m^2 which was escalated by 2.5 g/m^2 weekly to a maximum of 7.5 g/m^2. Dose escalation was not continued if a patient developed methotrexate toxicity, such as oral mucosal ulceration, nephrotoxicity (increase in serum creatinine of $> 50\%$), and myelosuppression with a total granulocyte count of $< 500/\text{mm}^3$, in spite of rescue with high-dose citrovorum factor at 150 mg/m^2. Such patients received subsequent courses of methotrexate at the highest doses not associated with significant toxicity. Initially, methotrexate was given weekly for 6 weeks and was then administered once every 3 weeks at the maximal dose that did not produce significant toxicity. All patients were hospitalized for methotrexate administration, which was given as an iv infusion in 1000 ml of 5% dextrose in water over 6 hours.

To maintain adequate urine flow before and after methotrexate administration and to avoid precipitation of methotrexate in the renal tubules, all patients were given sodium bicarbonate (1200 mg orally every 6 hours) starting 24 hours before methotrexate infusion and continuing for a total of 72 hours. In addition, all patients received acetazolamide (250 mg orally 1 hour before methotrexate administration) and iv furosemide (20 mg 1 and 6 hours after methotrexate infusion). Urine pH was checked every 6 hours; if it was found to be < 7.0, additional sodium bicarbonate or acetazolamide was given.

Immediately after methotrexate infusion, all patients received additional 24-hour iv fluids consisting of 1000 ml of normal saline plus 20 mEq of KCl plus 50 mEq of sodium bicarbonate every 6 hours. Citrovorum factor rescue (12 mg/m^2) was given 2 hours after cessation of methotrexate infusion and every 6 hours for 72 hours by the iv or im route. At 24 hours after methotrexate infusion, if the methotrexate blood level was $> 5 \times 10^{-5}$ mol/liter or the serum creatinine was $> 50\%$ above baseline, super-rescue with citrovorum factor at 150 mg/m^2 every 6 hours for 64 hours was instituted. A further 48 hours of citrovorum factor rescue at 12 mg/m^2 every 6 hours was given if the 48-hour methotrexate blood level was $> 1.0 \times 10^{-6}$ mol/liter, if the serum creatinine elevation was $< 50\%$ 24 hours after methotrexate infusion, or if there was any sign of methotrexate toxicity, such as sore mouth, oral ulcers, or skin rash.

A complete remission was defined as disappearance of all clinical evidence of active tumor for a minimum of 4 weeks, including normalization of osseous roentgenograms and absence of all tumor-related symptoms. A partial remission was defined as $\geqslant 50\%$ decrease in the sum of the product of the diameters of measured lesions, including complete and partial recalcification of osseous lesions radiologically. There could be no simultaneous increase in the size of any lesion or the appearance of new lesions. In the case of hepatic metastases with hepatomegaly that could only be measured unidirectionally, a 30% reduction in the size of the hepatomegaly was required. Stable disease was defined as a steady state of response which was less than a partial remission or as absence of increasing disease for at least 8 weeks. No new lesions could appear in this category. Increasing disease was defined as unequivocal increase of at least 25% in the size of any measurable lesions. The appearance of significant new lesions also constituted progressive disease. All patients were considered evaluable if they had received at least three doses of methotrexate. Patients with nonmeasurable disease were evaluable for toxicity only. The Gehan modification of the Wilcoxon test (17) was used to test differences between curves.

RESULTS

Thirty-nine patients were entered in the study. There was one early death (within 2 weeks of therapy) and one patient was lost to followup. Three patients were considered inevaluable for tumor response because of nonmeasurable disease and seven patients were disqualified because of inadequate trials of < 3 weeks. Among the 27 patients who were evaluable for response, there were one complete and seven partial responders, for an overall response rate of 29%. Response occurred at a median of 6 weeks after the start of treatment (range, 3–34 weeks). Of the 18 nonresponders, 13 had stable disease and three demonstrated increasing disease.

Among the 27 evaluable patients, 19 had received methotrexate (30 mg/m^2 on Days 1 and 8 every 21 days) in combination with cyclophosphamide and 5-FU (given as maintenance therapy), while eight patients had had no prior exposure to methotrexate. The response rates observed in these two groups of patients appeared to be similar: 31% for those who had prior exposure to methotrexate versus 25% for those who had no prior exposure. The response rates in individual organ sites are shown in table 1. There appeared to be no significant differences in response by site of metastases.

Table 2 shows the major patient characteristics by response status. There was no significant difference between responders and nonresponders in terms of age,

TABLE 1.—Response by site

Disease site	Total No. of patients	No. of patients responding (%)
Soft tissue	8	3 (37)
Lymph node	2	0
Lung	9	2 (22)
Pleura	2	1 (50)
Liver	4	1 (25)
Bone	14	3 (21)
Bone marrow	1	0

TABLE 2.—Patient characteristics by response status

	Responders	Nonresponders
Age (yrs)		
Median	50	51
Range	33–60	34–64
Disease-free interval (mos)		
Median	10	14
Range	0–35	0–64
Duration of prior therapy (mos)		
Hormonal therapy		
Median	8	4
Range	1–67	1–14
Chemotherapy		
Median	12	14
Range	2–45	5–28
Total duration of prior therapy		
Median	16	16
Range	4–79	5–35
Dominant disease site		
Soft tissue	0	4
Osseous	4	5
Visceral	4	10
No. of organ sites		
1	3	8
2	2	7
3	2	1
4	1	3

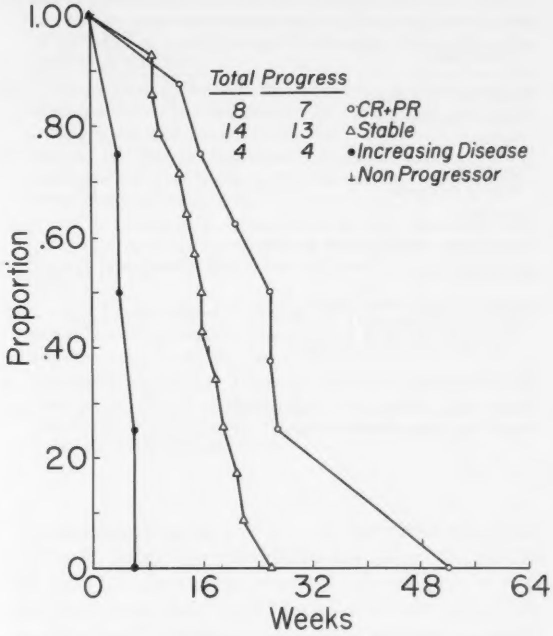

FIGURE 1.—Time to progression in patients receiving high-dose methotrexate.

menopausal status, dominant disease site, number of organ sites involved, or extent of prior chemotherapy. The median time from initiation of therapy to therapeutic failure for the responding patients was 26 weeks, compared to 16 weeks for those with stable disease ($P = 0.02$) and 4 weeks for those with increasing disease ($P < 0.01$ compared to responders; $P < 0.02$ compared to stable disease patients) (fig 1).

TOXICITY

Myelosuppression was mild, transient, and noncumulative (table 3). In general, the degree of myelosuppression observed did not appear to be dose-related. Thrombocytopenia (platelet count < 100,000/mm^3) occurred more often than neutropenia; the absolute granulocyte count was not < 500/mm^3 in any patient.

The nonhematologic toxicity is shown in table 4. Two patients became confused and disoriented within the first 24 hours after cessation of methotrexate infusion. A full metastatic workup for central nervous system involvement including spinal tap was negative in both cases, but the CSF methotrexate level 24 hours after methotrexate infusion was found to be 1×10^{-7} and 6×10^{-7} in both patients (18).

The incidence of gastrointestinal toxicity did not appear to be dose-related and was mild in most instances. Two patients developed an elevation in their serum creatinine to > 2 mg/100 ml, with a concurrent fall in their

TABLE 3.—Hematologic toxic effects

		Median lowest counts (cells × 10^3/mm^3)					
Methotrexate (g/m^2)	No. of evaluable courses	Wbcs (range)	Median Day	Absolute granulocytes (range)	Median Day	Platelets (range)	Median Day
2.5	27	4.6 (2.5–7.1)	5	3.0 (1.5–5.7)	5	210 (90–364)	6
5.0	30	4.0 (1.6–7.5)	5	2.9 (1.2–5.2)	5	194 (90–305)	6
7.5	54	3.8 (1.2–8.8)	6	2.6 (0.5–6.5)	6	220 (5–379)	6

TABLE 4.—Nonhematologic toxic effects in 135 evaluable courses

Toxic effect	% of courses with toxicity
Nausea and vomiting	
Mild to moderate	34
Severe	15
Stomatitis	
Mild to moderate	8
Severe	5
Fever (origin unknown) and neutropenia	2
Septicemia*	< 1 (1 patient)
Central nervous system effects	
Fatigue and lethargy	25
Confusion and disorientation	2
Allergic reactions*	< 1 (1 patient)
Renal (serum creatinine > 2 mg/100 ml + > 50% fall in creatinine clearance)	3

*1 death.

creatinine clearance of > 50% in spite of adequate hydration, diuresis, and alkalinization of urine. The first dose of methotrexate for both patients was 2.5 g/m^2 and the second dose was 5 g/m^2. Their renal function did not return to baseline levels upon cessation of methotrexate therapy. There were two drug-related deaths with this treatment program. One patient with neutropenia died from septicemia, while the other died from probable allergic reaction to methotrexate with central nervous system dysfunction and pneumonitis.

DISCUSSION

High-dose methotrexate with citrovorum factor rescue appears to be an active chemotherapeutic regimen for patients with advanced metastatic breast cancer. There was no significant difference between response rates in patients with and those without prior exposure to methotrexate at conventional doses. This suggests that high-dose methotrexate with citrovorum factor rescue may be useful as salvage therapy for patients who develop progressive disease after various regimens involving cyclophosphamide, methotrexate, and 5-FU in which methotrexate is given at standard doses.

Although the established clinical dictum is to not employ as salvage therapy a drug to which patients have been unresponsive, responses were observed even in patients who were refractory to conventional doses of methotrexate. This observation of a dose-related response and the available animal tumor data (19) indicate that further studies may be warranted to establish clearly the implications of this observation not only for methotrexate, but also for other available chemotherapeutic agents for which the dose-related response has not been well-defined clinically.

The 29% objective response rate observed in our patient population compared unfavorably with those rates reported in other studies. Schwarzenberg et al (20) reported a 50% response rate in 12 breast cancer patients who were treated with methotrexate (75 mg every 8 hours × 6 every 2 weeks). More recently, in an analysis of the total experience with high-dose methotrexate and citrovorum factor rescue in breast cancer, Bertino (21) reported an overall response rate of 38%. Unfortunately, there was no mention of the extent of prior chemotherapy and the duration of remission and survival in these patients. A review of the literature suggests that methotrexate in conventional doses produces a response rate of 20%–35% in breast cancer patients (7–11). Nevertheless, since none of these studies are directly comparable due to variations in patient selection, no conclusions can be made regarding the relative merits of high-dose methotrexate with citrovorum factor rescue versus conventional doses of methotrexate. Thus far, the response rate observed with the high-dose methotrexate/citrovorum factor rescue program does not appear to be better than that noted in earlier studies employing methotrexate in intermediate doses given sequentially with L-asparaginase (22) or in conventional doses in combination with vincristine (23). Aside from two drug-related deaths, the toxicity of high-dose methotrexate with citrovorum factor rescue appeared to be acceptable and comparable to that observed by other investigators (11–15,24). However, in view of the enormous cost of the large quantities of methotrexate required and the close pharmacologic monitoring necessitated by this approach, we do not feel further studies would be worthwhile.

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Phase II Study of *cis*-Dichlorodiammineplatinum(II) in Stage IVB Hodgkin's Disease[1,2]

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SUMMARY

A broad phase II study of *cis*-dichlorodiammineplatinum(II) was conducted. Eight patients with stage IVB Hodgkin's disease were studied. Four of these patients attained an objective partial remission of 7–19+ weeks' duration. The usual time to the occurrence of a ⩾ 25% response was 1 week. This drug is active in far-advanced Hodgkin's disease with a projected lower limit of response rate from 19% to 21%.

[Cancer Treat Rep 63:763–766, 1979]

cis-Dichlorodiammineplatinum(II) (CDDP) belongs to a new class of antineoplastic agents with demonstrated activity in man (1). Because of the need for continued identification of new therapies for patients with Hodgkin's disease not achieving long-term disease-free survival, we initiated a phase II trial with this agent in 1976. This trial included patients with a variety of disseminated malignancies. Eight of these patients had stage IVB Hodgkin's disease and are the subject of this report.

PATIENTS AND METHODS

A broad phase II trial was initiated using CDDP on an outpatient schedule which used vigorous hydration and mannitol-induced diuresis as follows: 500 ml/hour of 5% dextrose in ½ normal saline iv for 4 hours; 12.5 g of mannitol by iv push; 40 mg/m^2 of CDDP in 250 ml of normal saline over ½ hour; and 12.5 g of mannitol by iv push followed by 2½ hours of hydration at 500 ml/hour of 5% dextrose in ½ normal saline. Initially, this program was scheduled to be given weekly with escalation of the dose to 55 mg/m^2 after four doses. Due to dose-limiting myelosuppression observed in the first 20 patients (2), the program was modified in November 1976 to 40 mg/m^2 iv for 3 weeks, 2 weeks with no treatment, and, if no hematologic toxicity was observed, 55 mg/m^2 for 3 of 5 weeks for subsequent cycles. Patients 1, 2, and 3 (table 1) were treated with the first schedule; the last five patients were treated with the modified schedule.

Weekly observations included cbc, platelet count, creatinine, BUN, electrolytes (before and after infusion), urinalysis, and tumor measurements. Pre-treatment audiograms and bone marrow evaluations were obtained in all patients except one and were repeated when subjective complaints of 8th nerve damage or unexplained cytopenias occurred respectively.

The criteria of the Eastern Cooperative Oncology Group were used to assess response. A partial response (PR) could not be recorded without a ⩾ 50% decrease in the sum of the products of the bidimensional measurable disease.

Patient 6 was recorded as having a mixed response and was classified as a nonresponder (table 1). The total duration of response (table 1) was measured from the start of a ⩾ 25% decrease in the measurable disease to the start of progression. Progression was defined as a ⩾ 25% increase in the measurable disease.

All patients were treated according to the above protocol which had been approved by the University of Iowa

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Committee A (Human Investigation). Written informed consent was obtained from all patients.

RESULTS

The patient characteristics and response data are shown in table 1. Four of eight patients achieved an objective response, with the usual start of a $\geq 25\%$ response occurring 1 week after initiation of therapy.

All patients treated achieved some decrease in the sums of the products of the diameters of the bidimensional measurable disease. Patients were classified as nonresponders for the following reasons: (*a*) patients 2 and 7 achieved only a 46% and 44% decrease respectively in measurable disease; (*b*) patient 3 had a duration of response of only 3 weeks even though there was a 59% decrease in the measurable lesions; and (*c*) patient 6 developed a tumor-related esophageal ulcer requiring radiation in a previously unevaluated area in spite of a 51% decrease in the indicator lesions. It is quite possible that this ulcer did not indicate progression, but the patient was removed from the protocol at that point.

As in our initial report of this program, the dose-limiting toxicity remains myelosuppression (2). Six of the eight patients in this report experienced dose-limiting myelosuppression (requiring delay of treatment for granulocyte counts of < 1700/mm^3 or platelet counts of < 125,000/mm^3). Three of seven patients with bone marrow evaluations prior to treatment had Hodgkin's disease noted in the marrow. These three patients had significantly lower mean neutrophil nadirs (1100 versus 3507/mm^3; $P < 0.025$) and significantly higher mean platelet nadirs (192,000 versus 112,600/mm^3; $P < 0.01$) than the five patients without documented bone marrow involvement. The apparent difference in myelosuppression is not evident if the percent change from the initial neutrophil or platelet count is examined. The mean percent change of the neutrophil counts was $-68\% \pm 15\%$ in the marrow-positive group and $-41\% \pm 33\%$ in the marrow-negative group; the changes in the platelet counts were $-56\% \pm 11\%$ and $-64\% \pm 18\%$ respectively.

The other toxic effects noted with this program have been more adequately described in 46 of the broad phase II trial patients who have been previously reported (3). Only one of these 46 patients experienced dose-limiting nephrotoxicity; this patient developed a transient elevation of the serum creatinine from 1.2 to 2.0 mg/100 ml. Nausea and vomiting requiring frequent antiemetics occurred in 19 of 46 patients, and two of 46 patients required electrolyte replacement while in the hospital. The eight patients described in this paper did not differ from the larger series in these respects.

One of the patients in the present series (patient 5) refused further therapy after an objective PR of 10+ weeks due to gastrointestinal toxicity, thus terminating treatment in this study. At that time the followup was

TABLE 1.—Patient characteristics and response data in Hodgkin's disease

Patient No.	Histology*	Initial Karnofsky score (%)	Response category	Time to occurrence of $\geq 25\%$ response (wks)	Maximum response (% decrease)	Duration of response (wks)	Prior polychemotherapy programs of curative intent†			Agents previously received‡
							MOPP	ABVD	Other	
1	MC	50	PR	1	99%	14	No	No	No	A, Alk, B, DTIC, P, Pcz, V
2	MC	50	No response	1	46%	4	Yes	Yes	No	A, Alk, B, DTIC, P, Pcz, V
3	LD	70	No response	2	59%	3	Yes	Yes	No	A, Alk, B, DTIC, Nu, P, Pcz, V, XRT
4	NS	70	PR	1	79%	19	Yes	No	Yes	A, Alk, B, DTIC, Nu, P, Pcz, V, XRT
5	NS	70	PR	2	60%	10+	Yes	No	Yes	A, Alk, B, I, Nu, P, Pcz, V, XRT
6	NS	90	No response	8	51%	5	Yes	Yes	No	A, Alk, B, I, Nu, P, Pcz, V, XRT
7	LD	70	No response	1	44%	5	Yes	Yes	No	A, Alk, B, Nu, P, Pcz, V, XRT
8	NS	60	PR	1	67%	10	Yes	No	Yes	A, Alk, B, Nu, P, Pcz, V, XRT

*MC = mixed cellularity; NS = nodular sclerosis; LD = lymphocyte depleted.

†Polychemotherapy programs: ABVD = Adriamycin, 25 mg/m^2 iv, Days 1 and 14; bleomycin, 10 units/m^2 iv or im, Days 1 and 14; vinblastine, 6 mg/m^2 iv, Days 1 and 14; and DTIC, 150 mg/m^2 iv, Days 1–5 or 375 mg/m^2 iv, Days 1 and 14. MOPP (in 28-day cycles) = mechlorethamine, 6 mg/m^2 iv, Days 1 and 8; vincristine, 1.4 mg/m^2 (maximum dose, 2.0 mg) iv, Days 1 and 8; procarbazine, 100 mg/m^2 orally, Days 1–10 or 1–14; and prednisone, 40 mg/m^2 orally, Days 1–10 or 1–14.

‡A = Adriamycin; Alk = alkylating agents; B = bleomycin; I = ICRF-159; Nu = nitrosoureas; P = glucocorticoids; Pcz = procarbazine; V = vinca alkaloids; XRT = radiation therapy.

TABLE 2.—Response of lymphoid malignancies to CDDP

Disease	No. of— Patients	No. of— Objective responses	Author(s) (ref No.)
Hodgkin's lymphoma	1	0	Lippman AJ, et al (4)
	2	1	Rossof AH, et al (5)
	8	4	Corder MP, et al (this report)
Non-Hodgkin's lymphoma	5	3	Talley RW, et al (6)
	2	0	Rossof AH, et al (5)
	14	4	Hill JM, et al (7)
	4	1	Wiesenfeld M, et al (3)
Chronic lymphocytic leukemia	3	0	Hill JM, et al (7)
Lymphoma, not specified	5	2, "useful"	Hayes D, et al (8)
	3	2	Hill JM, et al (9)

less rigorous and the precise duration of response could not be ascertained. With the exception of this patient, progression of disease occurred while the patients were receiving treatment.

DISCUSSION

Table 2 summarizes the existing phase II data for Hodgkin's disease and non-Hodgkin's lymphoma treated with CDDP with a variety of doses and schedules.

It is clear from our study that CDDP has substantial activity when administered in this fashion. If a binomial distribution of responses is assumed, then the *lower* limit of activity would be a PR rate of 19% with the one-sided confidence limit of 95%. If a Gaussian distribution is assumed, then the *lower* limit would be a PR rate of 21% with the one-sided confidence limit of 95%. This confidence interval provides a measure of minimal response that can be obtained. It includes the effect of the number of patients as well as the variability associated with the estimate. In fact, the single point that best estimates response is $4/8 = 0.5$ which has a standard error of 0.18.

Seven of the eight patients studied and three of the four patients with objective responses had received prior mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) chemotherapy as shown in table 1. In addition, seven patients had received either Adriamycin, bleomycin, vinblastine, and DTIC (ABVD) or other prior polychemotherapy programs of curative intent. All patients had been heavily previously treated with programs that were administered ordinarily over a period of years prior to the start of CDDP therapy. In all cases, the agents that these patients were resistant to included anthracycline antibiotics, alkylating agents, glucocorticoids, procarbazine, and vinca alkaloids. All patients had received bleomycin, but were not necessarily resistant to the drug. Our data would suggest, but not establish, a lack of cross-resistance with CDDP with these other classes of agents. It is possible that some of the patients retained sensitivity to individual components of the polychemotherapy programs while demonstrating failure on the program as a whole. We do not feel that this possibility detracts from the significance of the high degree of activity observed in these heavily treated patients with advanced Hodgkin's disease. The rapid occurrence of a $\geqslant 25\%$ response in seven of eight patients studied (median time, 1 week) indicates that CDDP might be particularly useful in patients with life-threatening organ dysfunction as a single agent or in combination. CDDP should be considered as a component of either initial polychemotherapy programs or at least earlier "salvage" therapies for patients failing to respond to MOPP.

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Cytokinetic Chemotherapy Design for the Treatment of Advanced Lung Cancer[1,2]

Marc J. Straus[3,4,*]

SUMMARY

Sixty patients with lung cancer, 48 with extensive disease and 12 with regional disease, were treated with cyclophosphamide and methotrexate on a schedule based on cellular kinetics concepts. Initial therapy was with cyclophosphamide (1.1 g/m^2 iv) followed by methotrexate (20 mg/m^2 orally twice weekly) beginning 9 days later when the tumor was considered to be most susceptible to an S-phase-specific drug. The overall objective response rate was 62% (25% complete responses and 37% partial responses) with an estimated median survival time (MST) of 46 weeks. Seventeen of 19 patients with small cell carcinoma (89%) responded (ten complete responses and seven partial responses). The MST was 62 weeks. The last nine patients entered in the study with small cell carcinoma had an MST of 71 weeks, reflecting additional responses to subsequent treatment. The objective response rate of large cell carcinoma (nine of 16 patients) and adenocarcinoma (ten of 18 patients) was 56%. The MST of patients with the former cell type was longer (57 weeks) than that of patients with the latter cell type (34 weeks). One of seven patients with epidermoid carcinoma responded. The MSTs of patients with a complete response and those with regional disease were 70 and 63 weeks respectively. Patients with a performance status of 0 or 1 survived longer (MST, 56 weeks) than those with a performance status of 2 or 3 (MST, 29 weeks). The mean dose of cyclophosphamide per course was 1.275 g/m^2 and the mean nadir leukocyte count per patient was 2890/mm^3. The incidence per course of leukocytes < 1000/mm^3 or platelets $< 50{,}000$/mm^3 was $< 3\%$. Mucositis was common. This schedule provides excellent maintenance therapy without undue toxicity. These survival time distributions compare favorably with those of previous reports, particularly for patients with small cell or large cell carcinoma, regional disease, or complete responses.

[Cancer Treat Rep 63:767–773, 1979]

The treatment of unresectable lung cancer has changed rapidly in recent years and now frequently includes combination chemotherapy. A number of drug combinations produce objective response rates > 50% (1). Small cell carcinoma is the most responsive cell type, with several combinations producing responses > 75% and median survivals of 9–12 months.

With the advent of more complicated and often more toxic regimens there is a need to focus on the optimal use of existing drugs. The cytokinetic and pharmacokinetic elements available for the rational design of treatment schedules have often been ignored.

Several years ago, a protocol was designed for the treatment of lung cancer which attempted to incorporate certain cytokinetic principles as well as special dose-response parameters of the drugs (2). The protocol included treatment with cyclophosphamide (CYC) and methotrex-

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TABLE 1.—Schedule of CYC/MTX therapy

	Cycle 1*					Cycle 2				
	1	9	13	16	20	22	30	34	37	41
	(days of treatment)					(days of treatment)				
CYC (1.1 g/m^2 iv)†	×					×				
MTX (20 mg/m^2 orally)†,‡		×	×	×	×		×	×	×	×

*Folic acid (1 mg) is administered daily on Days 1–5 of every course.
†Initial drug dose.
‡Maximal single dose of MTX = 37.5 mg.

ate (MTX). The rationale has been described in a publication which summarizes the clinical data for the first 28 patients studied (2). The treatment scheme is shown in table 1. In brief, it was hypothesized that the high initial dose of CYC would reduce the tumor cell population and that maximum recruitment of cells into S phase would occur approximately 8 days later, at which time the S-phase-specific drug, MTX, would be given. As a non-phase-specific drug, CYC is best administered on a high-dose intermittent schedule (3). MTX was given twice weekly, consistent with the mean cell cycle time of human lung cancer (4). An animal model system had been designed which supported some of these hypotheses (5).

A carefully structured dose-modification scheme for each drug maximizes the amounts of drugs given with acceptable toxicity.

The results in the first 28 patients (26 with extensive disease) were a 71% objective response rate with an estimated median survival time (MST) of 48 weeks. The best results were noted in small cell carcinoma (ten responses among 11 patients) and large cell carcinoma (five responses among seven patients).

The first 60 consecutive evaluable patients are reported. The study now permits a more detailed evaluation of response and survival by cell type, performance status (PS), extent of disease, and previous treatment with radiation. The series now includes 12 patients with regional disease. An analysis of hematologic toxicity has provided special insights into dose-modification capabilities with these drugs and the potential for upgrading this and other treatment schedules.

PATIENTS AND METHODS

From December 1971 to June 1977, 62 patients with unresectable lung cancer were entered in this protocol. Two patients were excluded from the analysis. One patient died 9 days after treatment began of an apparent myocardial infarction, and a second patient was not treated according to the protocol, although survival was 59 weeks. Patients initially entered in the study had extensive disease. Subsequently, patients with regional disease were also accepted. Survival data were analyzed as of September 30, 1978. The median followup periods for patients with small cell and non-small cell cancers are 178 and 155 weeks respectively.

Criteria for admission to the study and pretreatment workup were as previously described (2). It is pertinent to reiterate that no patients had received prior chemotherapy and patients with cerebral metastases or a PS of 4 were excluded. Destructive radiographic bone changes or positive scans were accepted as evidence of dissemination. Except in six cases with both multiple large defects on liver scan and abnormal liver chemistries, these tests necessitated percutaneous needle biopsy for confirmation of metastatic disease. A liver biopsy was positive in six of ten patients suspected of having liver metastases. A positive node biopsy or aspirate, brain scan, or bone marrow biopsy was considered to be evidence of dissemination. The latter was positive in three of 15 patients with small cell carcinoma and two of seven patients with large cell carcinoma.

The Eastern Cooperative Oncology Group performance status scale (0–4) was used. The standard staging classification was used (6). Chemotherapy consisted of two-drug combination therapy, as outlined in table 1. CYC was administered iv every 3 weeks. The initial dose was 1100 mg/m^2 and was given over 10–30 minutes. MTX was administered orally twice weekly in four doses beginning on the ninth day of each 21-day course at an initial dose of 20 mg/m^2 (not > 37.5 mg/dose).

The dose-modification schemes were previously described (2). The doses of CYC and MTX are modified according to both the wbc and platelet counts and this requires use of four separate tables. The most important relationship is that of the CYC adjustment according to the wbc count (table 2)[5] since CYC produces more leukopenia than thrombocytopenia. The table indicates that nadir wbc counts from the previous course are used to adjust the CYC dose. If in the prior course the wbc count was < 2000/mm^3, then CYC is reduced 10%–20%. How-

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[5] Table 2 is the dose-modification scheme in current use which results in more aggressive treatment than these 60 patients received. See previous report (2) for original dose modifications.

TABLE 2.—Dose modifications for CYC*

Lowest wbc count on Day 7 or 14 of previous course (cells/mm^3)	Wbc count on Day 1 of new course (day of CYC administration)	Dose adjustment
1400–2000	> 2500	No change
500–1400	> 2500	Decrease 10%
< 500	> 2500	Decrease 20%
> 3000	1500–2500	Decrease 10%
< 3000	1500–2500	Decrease 30%
> 3000	1000–1500	Decrease 30% †
< 3000	1000–1500	Decrease 60%
	< 1000	Omit dose‡
2000–2500	> 2500	Increase 5%
2500–3000	> 2500	Increase 10%
> 3000	2500–3000	Increase 15%
> 3000	> 3000	Increase 20%

*Original m^2 measurement is used throughout the study for all dose calculations.

†When a dose of drug is reduced by 30% or 60%, the next dose of drug is re-instated at 80% of the offending dose if the wbc count is ≥ 3000/mm^3 and the platelet count is ≥ 100,000/mm^3. The next dose is re-instated at 90% of the offending dose if the wbc count is ≥ 4000/mm^3 and the platelet count is ≥ 100,000/mm^3.

‡ If the dose is omitted, wait until the wbc count is > 3000/mm^3 and the platelet count is > 100,000/mm^3 before re-starting and treat at 80% of the last dose.

ever, if on the day of CYC treatment the wbc count is < 3000/mm^3, greater reductions in CYC doses are made. Increments of CYC doses are small: 5%–20%. An important provision is the re-escalation of CYC dose after a dose decrease (table 2, *see* footnotes). Approximately 15% of the patients had a nadir wbc count near the time of re-treatment. Retrospective analysis demonstrated that this group of patients received less CYC and yet had the same mean nadir wbc count as the patients whose nadirs occurred 1 or 2 weeks after CYC. Therefore, an adjustment was made in table 2 after analysis of these 60 patients which allowed a higher dose of CYC to be given provided the interim wbc counts during Weeks 1 and 2 were ≥ 3000/mm^3. We have noted little or no additional toxicity since making this change in the schedule.

The MTX modifications were weighted more heavily to thrombocytopenia than to leukopenia but it was rarely necessary to adjust the MTX dose based on blood cell counts. MTX was also adjusted for impaired renal function. For creatinine levels of 1.6–2.5, 2.6–3.5, and > 3.5 mg/100 ml, the dose of MTX was 67%, 33%, and 0 respectively. MTX was omitted when stomatitis occurred. If the stomatitis occurred before the fourth dose of MTX or if the stomatitis was severe, MTX dose was reduced by 2.5 mg/dose for subsequent courses. After three consecutive courses with no stomatitis, MTX was re-escalated by 2.5 mg/dose/course to a maximum of 37.5 mg/dose.

Folic acid (1 mg) was administered for the first 5 days of each course to the last 45 patients entered in the study. It was hoped that the folate stores could be replenished when MTX was not being given, thereby reducing the toxicity of MTX.

The patients had weekly cbcs and platelet counts. Tumor measurements, weight, and PS were evaluated every 3 weeks and blood chemistry values were determined as above. Bone and liver scans were repeated every 3 months.

The standard criteria for complete response (CR) and partial response (PR) were used (2). For most of the first 30 patients entered in the study, treatment was discontinued in the presence of progressive disease. Subsequently, most of the patients, and all of those with small cell carcinoma, have been treated with other drugs. Several patients received a combination of Adriamycin and procarbazine with or without VP-16-213 and vincristine and over half of these patients had further objective responses. In some cases, additional chemotherapy was given after the second response. The survival time was calculated from Day 1 of CYC and MTX treatment to death.

RESULTS

Sixty of the 62 patients consecutively entered in this study were evaluable. A summary is shown in table 3. There were 49 males and 11 females. Thirty-eight (63%) of the patients had a good performance status (0 or 1). Eighty percent of the patients had documented extensive disease and 16 (27%) of the patients had received prior radiotherapy. The location of metastatic disease by cell type is presented in table 4. The most common location of clinical metastatic disease at the beginning of treatment was bone (43%). Approximately 20% had liver metastases and 20% had nodal metastases. Five of six patients with contralateral lung disease had adenocarcinoma.

A summary of the survival data is shown in table 5 (7). Overall, the response rate was 62% (25% CRs and 37% PRs), with an estimated MST of 46 weeks (table 5). Four patients were still alive at the time of this writing.

The best results were achieved in small cell carcinoma. There were 17 responses among 19 patients (89%) with ten CRs (53%) and an MST of 62 weeks. There were nine responses among the 16 large cell patients (three CRs and six PRs), with an MST of 57 weeks. The response rate of the adenocarcinoma patients was similar (ten of 18 patients) but remission duration was decreased as was survival time, with an MST of 34 weeks. One of the seven epidermoid patients responded and the MST was 36 weeks. The median durations of response were 39 and 32 weeks for the small cell and non-small cell patients respectively.

The 15 patients with a CR had a significantly longer MST (70 weeks) than patients with either a PR (45 weeks) or no response (31 weeks) ($P = 0.006$) (8). For the CR patients, the small cell group had an MST of 67 weeks compared to 77 weeks for the non-small cell group; this difference was not significant. For the PR patients, the MSTs were 49 and 45 weeks respectively (not significant). The median duration of response was 50 weeks for the CR patients: 50 weeks for small cell patients and 59 weeks for non-small cell patients (not significant). The median duration of response was 21 weeks for the PR patients: 20 weeks for small cell patients and 22 weeks for non-small cell patients (not significant). The duration of response for small cell CRs was significantly longer than that for small cell PRs ($P = 0.01$).

Similarly, the PS markedly influenced survival: 56 weeks for patients with PS 0 or 1 and 29 weeks for pa-

TABLE 3.—Distribution of patients by sex, cell type, PS, and extent of disease

	All patients	Patients with— Small cell carcinoma	Large cell carcinoma	Adeno-carcinoma	Epider-moid carcinoma
All patients	60	19	16	18	7
Males	49	14	12	16	7
Females	11	5	4	2	0
Patients with—					
PS 0 or 1	38	16	10	8	4
PS 2 or 3	22	3	6	10	3
Regional disease	12	4	6	1	1
Extensive disease	48	15	10	17	6
Prior radiation therapy	16	2	3	5	6
No prior radiation therapy	44	17	13	13	1

TABLE 4.—Location of metastatic disease

Location of metastasis*	All patients	Patients with— Small cell carcinoma	Large cell carcinoma	Adeno-carcinoma	Epi-dermoid carcinoma
Bone	26	6	7	9	4
Liver	12	5	1	6	0
Bone marrow†	5	3	2	0	0
Node	13	4	4	3	2
Opposite lung	6	0	1	5	0

*Patients with extensive disease. Some patients had multiple locations of metastatic disease.

†Not performed in all patients.

TABLE 5.—Summary of response to CYC/MTX therapy for lung cancer

Cell type	No. of patients	No. of patients with— CR	PR	Stable disease	Progression	No. of patients alive	Estimated MST (wks)
Large cell	16	3	6	6	1	3	57
Adenocarcinoma	18	2	8	5	3	1	34
Small cell	19	10	7	2	0	0	62
Epidermoid	7	0	1	3	3	0	36
	60	15	22	16	7	4	46

tients with PS 2 or 3 ($P = 0.0001$). For the PS 0 or 1 patients, the MST of the small cell patients (69 weeks) was not significantly longer than that of the non-small cell patients (52 weeks). The MSTs of the PS 2 or 3 patients were 29.5 weeks for small cell patients and 28 weeks for non-small cell patients. Patients with regional disease had an MST of 63 weeks: 69 weeks for small cell patients and 61 weeks for non-small cell patients (not significant). In patients with extensive disease, the MST was 43 weeks: 62 weeks for small cell patients and 38 weeks for non-small cell patients ($P = 0.07$). The MST was 52 weeks for patients without prior radiation therapy compared to 33 weeks for those who had received radiation therapy ($P = 0.008$).

A subgroup with an excellent outcome was the group of nine patients with regional disease who had objective responses (four CRs and five PRs). Two patients were alive at the time of this writing and the MST was 76 weeks. The 28 responders with extensive disease had an MST of 53 weeks. The response rate for the PS 0 or 1 group was 76% compared to 36% for the PS 2 or 3 group. The MSTs of the responders in the PS 0 or 1 and the PS 2 or 3 groups were 68 and 38 weeks respectively.

The 19 patients with small cell carcinoma were further divided into the first 10 and the last nine entered in the protocol. The MST was 56 weeks for the first group and 71 weeks for the second group ($P = 0.11$). The median duration of response was equal for both subgroups.

The survival of patients with large cell carcinoma was better than that of patients with adenocarcinoma. However, in the adenocarcinoma group there was the highest percentage of patients with a PS of 2 or 3 (56%) and the lowest percentage of patients with regional disease (6%) (table 3). Therefore, the MST was compared in these two cell types by inclusion of only the patients with PS 0 or 1 and/or regional disease. The MST of the large cell subgroup (11 patients) was significantly longer (72 weeks) than that of the nine patients in the adenocarcinoma subgroup (45 weeks) ($P = 0.01$), which suggests that large cell carcinoma indeed responds better to this therapy than does adenocarcinoma. The comparable 16 small cell patients had an MST of 68 weeks which is not significantly different from the survival of the large cell patients.

An objective response was evident within 2–6 weeks in 25 patients, 7–9 weeks in seven patients, and 10–12 weeks in five patients.

The objective response rates by disease site were as follows: bone marrow, 80%; nodes, 77%; intrathoracic disease, 62%; liver, 42%; and bones, 19%.

CNS involvement was documented in three patients with small cell carcinoma. The survival times of these three patients at the time of recurrence was 64–96 weeks, and CNS involvement occurred after progression was documented elsewhere.

The average dose per 3-week course of CYC administered was 1.275 g/m^2 and the initial dose was 1.1 g/m^2 using the dose-modification scheme previously reported (2). Therefore, in most patients it was possible to increase the dose of CYC and maintain a higher dose level. The highest average dose achieved was 1.49 g/m^2/course and the lowest was 0.69 g/m^2. There was no apparent relationship between the average dose of CYC and the response rate or survival.

The toxicity was evaluated in 309 treatment courses which represents the last half of the patients entered in the study. In some of the earlier patients, the dose increments were not as great (2). The mean nadir wbc count per patient was 2890 ± 110 (SE)/mm^3.

In the patients who developed their nadir leukopenia on Week 3, ie, the day of scheduled CYC re-treatment, the average dose of CYC was only 0.82 g/m^2 and the mean nadir wbc count per patient was 3000/mm^3, similar to that of the other patients. Therefore, this group of patients had similar toxicity but received far less drug. Accordingly, adjustments were recently made to increase the dose of CYC in this group of patients (table 2). There was not a statistically significant relationship between the mean nadir wbc count and survival.

The incidence of < 1000 or < 2000 wbcs/mm^3/course was 2.2% and 14.2% respectively (table 6). Only one patient was admitted for fever and possible infection secondary to this protocol. He developed a septicemia and recovered after the use of appropriate antibiotics. The incidence of $< 50{,}000$ or $< 100{,}000$ platelets/mm^3/course was 2.6% and 8.7% respectively, with no platelet count falling to $< 25{,}000$/mm^3 and no episodes of bleeding. The incidence of wbc counts < 2000/mm^3 was significantly higher ($P < 0.05$) in the PS 2 or 3 group (24%) compared to the PS 0 or 1 group (12.3%). There was no difference in the incidence of platelet counts $< 100{,}000$/mm^3 in PS 0 or 1 versus PS 2 or 3 groups. Similarly, there was no obvious relationship between the extent of the disease and the hematologic toxicity (table 6).

Hemorrhagic cystitis did not occur. Mild mucositis occurred in at least half of the patients and frequently required dose adjustments more than once. Severe mucositis with inability to eat occurred in three patients. Mucositis almost always resolved before the next course of MTX began.

Mild alopecia occurred in most patients with total alopecia occurring in approximately one fourth. Nausea frequently occurred and vomiting was less frequent in the first 24 hours after CYC. These symptoms were often controlled by antiemetics. Episodes usually lasted < 2 hours and did not recur predictably in a given patient.

In two patients, there were transient elevations of SGOT and LDH to two to three times normal, which was probably related to MTX hepatic toxicity. The MTX was continued without further problems.

This regimen was well-tolerated. It necessitated one clinic visit every 3 weeks. Most interim blood cell counts

TABLE 6.—Hematologic toxicity of CYC/MTX based on 309 3-wk treatment courses

	No. of courses	No. of courses with wbc count $< 1000/\text{mm}^3$ (%)	No. of courses with wbc count $< 2000/\text{mm}^3$ (%)	No. of courses with platelet count $< 50{,}000/\text{mm}^3$ (%)	No. of courses with platelet count $< 100{,}000/\text{mm}^3$ (%)
All patients	309	7 (2.2)	44 (14.2)	8 (2.6)	27 (8.7)
Patients with—					
PS 0 or 1	259	5 (1.9)	32 (12.3)	8 (3.0)	24 (9.3)
PS 2 or 3	50	2 (4.0)	12 (24)	0 (0)	3 (6.0)
Regional disease	114	1 (0.9)	15 (13.1)	4 (3.5)	14 (12.2)
Extensive disease	195	6 (3.1)	29 (14.9)	4 (2.1)	13 (6.7)

were obtained at community laboratories. Only two patients discontinued therapy because of side effects. In both cases, there was an unusual amount of mucositis and nausea associated with the MTX, and after 11 and eight courses of CYC/MTX respectively, hydroxyurea was substituted for the MTX.

There was no obvious amelioration of the frequency or severity of mucositis in the patients who received folic acid as compared to those who did not. Moreover, there was no apparent difference in response rate. However, the group without folic acid was small (15 patients) and not entirely comparable to the latter group. We have continued the administration of the folic acid.

Several of the patients entered in the study more recently, particularly those with small cell and large cell carcinoma, received subsequent chemotherapy after disease progression. The regimen often was combination Adriamycin and procarbazine with or without VP-16-213 and vincristine with several patients responding (Straus MJ, unpublished data).

DISCUSSION

The overall objective response rate of 62% is similar to that noted in a previous report (71%) (2). The overall MSTs in these reports were 46 and 48 weeks respectively.

The best response rate was in small cell carcinoma, 89% with a 53% CR rate. The MST has increased from 51 weeks in the previous report to 62 weeks in the current report. This increase is best demonstrated by comparing the results of the first ten patients to those of the last nine patients. One difference in the two groups has been that no patients with regional disease were included in the early group while four patients with regional disease were included in the latter group. More important, few of the early patients received subsequent treatment after CYC/MTX failure. All of the patients in the latter group who progressed were treated with alternative therapy. Progression following a response was defined as any measurable worsening of the lesion or the development of any new lesion. These patients therefore had other treatment and often responded. The mean duration of response to CYC/MTX in the two groups was identical.

The 62-week survival in the total small cell carcinoma group compares favorably with other published reports (9–11) and suggests that mean survivals > 1 year with nontoxic regimens are achievable. It is important to point out that the PS of the patients with small cell carcinoma was generally good (table 3). In both our study and that of Israël et al (9), patients with small cell carcinoma achieving a CR lived approximately 1.5 years.

The patients with large cell carcinoma had a response rate of 56%. The MST was 57 weeks, which represents a particularly long survival for this cell type (1). There were, however, six patients in this group with regional disease, including five of the last nine patients entered in the study. The survival of these patients with a good prognosis, ie, PS 0 or 1 or regional disease, was as good as the comparable small cell group.

The overall response rate for adenocarcinoma was similar (56%) but the MST was only 34 weeks. Although more of these patients had a poor performance status and extensive disease, as compared to the group with large cell carcinoma, the MST of the patients with adenocarcinoma with PS 0 or 1 or regional disease was still only 45 weeks.

The patients with adenocarcinoma who seemed to do particularly poorly were those with massive pleural effusions or multiple bilateral lung lesions. These occurrences were almost pathognomonic of adenocarcinoma. Five of six patients with bilateral lung involvement had this cell type (table 4).

Although there were few patients with epidermoid carcinoma, the response rate was obviously poor. In this group, however, six of seven patients had failed previous radiation therapy and the efficacy of the treatment in previously untreated patients is conjectural. Other recent studies have demonstrated a better response rate than ours in epidermoid lung cancer (1,12,13) and many of these studies have included the use of Adriamycin.

These data demonstrate the important survival differences which occur in the different PS groups (14). The MSTs of the PS 0 or 1 group and the PS 2 or 3 group were 56 and 29 weeks respectively. The MST of patients with regional disease was 63 weeks. This is particularly impressive inasmuch as no radiation therapy was given to this group.

In the subgroup of 12 regional disease patients there were nine responders with an MST of 76 weeks; patients are still alive as of this writing. Only four of the 12 patients with regional disease had small cell carcinoma (table 3). The MST of all patients with extensive disease was 43 weeks (53 weeks for the responders). Prior radiation therapy seemed to have exerted a negative effect on survival.

The disease sites which responded the best were bone marrow, nodes, and primary disease. Bone marrow biopsies were done in few patients with non-small cell carcinoma. The response rate therefore is based mostly on small cell patients. Only four of 15 of the CR patients had bone involvement. Disease in this location has the lowest response rate.

The carefully regulated dose scheduling of this protocol induces an almost consistent level of leukopenia in each patient with few episodes of severe leukopenia. This is achieved with high levels of CYC. In $> 80\%$ of the courses the peak wbc toxicity was noted when the 2-week blood cell count was determined. Approximately 15% of the patients reach their nadir leukopenia at the Week-3 count. Accordingly, we now consider that the first-week count in this study may be unnecessary.

The group with nadir wbc counts during Week 3 received less CYC with similar leukopenia. Currently, we have begun to increase CYC doses in this group provided that the Week-2 counts are adequate.

No relationship could be established between mean nadir leukopenia and survival. The variation in leukopenia was probably too small to establish a difference. However, there was also no relationship between the mean dose of CYC/course/m^2 and survival, and in this case the variation in dose was substantial. This suggests that either there were insufficient data at this point or that there may be some correlation of normal cell and tumor cell responsiveness to the drug. That is, patients whose bone marrow seems more sensitive to the drug and who therefore receive less drug may have a more sensitive tumor cell population. It is possible that these patients have higher levels of active metabolites per dose of drug and therefore achieve a greater effect on normal and tumor populations per dose. It would be interesting to test this concept.

Mucositis was common but almost never severe. Patients seem to exhibit very individual sensitivity to MTX and there seems to be some cumulative effect in developing this toxicity. This treatment schedule was designed to titrate MTX so that early mucositis would occur.

Other toxicity was generally quite well-accepted and the program was only interrupted in two patients because of intolerance.

Despite the rigorous system we employ to assure protocol compliance, there were a few protocol violations in this study. Almost all of the errors occurred in not properly increasing the dose of CYC after it had been reduced by $\geq 33\%$. This protocol is complicated to follow and in our experience the tables must be available each time a dose decision is made.

The effect of the cytokinetic scheduling on the outcome is still unclear. Better definition of the cytokinetics in human cancers will hopefully provide a better basis for schedule design (15). It does seem clear, however, that carefully designed dose modifications permit much higher doses of CYC with acceptable toxicity. This approach to treatment design has application to other diseases and other drugs.

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Determination of *N*-(Phosphonacetyl)-L-aspartic Acid (PALA) in Dog Serum and Urine by Gas Chromatography and Selected Ion Monitoring [1,2]

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SUMMARY

Methods are described for quantitating *N*-(phosphonacetyl)-L-aspartic acid (PALA), a new antitumor agent presently undergoing clinical evaluation. These methods were developed to measure this compound in serum by gas chromatography using the selectivity of a nitrogen-phosphorus detector and in serum and urine by the gas chromatographic/mass spectrometric technique of selected ion monitoring. PALA and the internal standard, *N*-(phosphonacetyl)-L-glutamic acid (HOMOPALA), were analyzed as their tetramethyl derivatives. Extraction from the biologic fluids was achieved by first precipitating the protein with acetone and then drying the serum with subsequent washes of acetone and diethyl ether. PALA and HOMOPALA were then extracted from the dried serum into an anhydrous HCl/methanol/tetrahydrofuran solution. After removing the solvent, the extract was reacted with diazomethane to form the tetramethyl derivatives. The feasibility of the method was demonstrated in the dog by comparing the total radioactivity with PALA equivalents determined in blood and urine samples following iv administration of PALA-^{14}C. The range of the analysis was 20.0–200 μg/ml for the urine samples and 0.5–15 μg/ml for the serum samples.

[Cancer Treat Rep 63:775–780, 1979]

N-(Phosphonacetyl)-L-aspartic acid (PALA), an analog of the transition state intermediate of the reaction catalyzed by aspartate transcarbamylase (1), is a potent inhibitor of this enzyme both in vitro (1) and in vivo (2). PALA is highly active against certain solid murine tumors (3) and is presently undergoing evaluation for antitumor effectiveness in man. To aid in the clinical evaluation of PALA we undertook the development of analytic methods to quantitate PALA in serum and urine. This report describes two techniques: (*a*) a gas chromatographic (GC) method using the selectivity of a nitrogen-phosphorus detector, and (*b*) a GC/mass spectrometric technique using selected ion monitoring (SIM). Application of these techniques to the quantitation of PALA in biologic extracts was demonstrated by comparing serum and urine levels of PALA in the dog determined analytically with values obtained from radioactivity measurements using PALA-^{14}C. Presently, the methods are being used to quantitate PALA in patient serum and urine.

MATERIALS AND METHODS

Reagents

Reference PALA and PALA-^{14}C (32.0 microcuries/mmol) were supplied by Stanford Research Institute, Calif (under contract N01-CM-33723 from the Division

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[3] Laboratory of Chemical Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md.

[4] Arthur D. Little, Inc, Cambridge, Mass.

[5] We would like to express our thanks to Dr. C. L. Patlak, National Institute of Mental Health, for the nonlinear least-squares analysis of our data.

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of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare). *N*-(Phosphonacetyl)-L-glutamic acid (HOMOPALA), used as the internal standard for quantitation (180 μg/ml in H_2O), was prepared from phosphonacetic acid and the dibenzyl ester of L-glutamic acid.[6] The compound was identified by mass spectrometry and elemental analysis. Saturated HCl in methanol (3 M as determined by base titration) was prepared by passing anhydrous HCl gas through a drying tower containing concentrated H_2SO_4 and then bubbling the HCl through absolute methanol. Diazomethane in chloroform, used for derivatization of PALA and HOMOPALA, was generated by the method of Fales et al (4). *N*-Methyl-*N*-nitroso-*N'*-nitroguanidine (Aldrich Chemical Co, Milwaukee, Wis) was used as the reagent to generate the diazomethane. The scintillation solution for radioactive counting was prepared by adding 6 g of 2,5-diphenyloxazole and 200 mg of *p*-bis[2-(5-phenyloxazolyl)]benzene (New England Nuclear Corp, Boston, Mass) to 1400 ml of toluene and 600 ml of methanol. All solvents were ACS reagent grade.

Animal Studies

Two female dogs (17 kg) were anesthetized with iv sodium pentobarbital and anesthesia maintained by intermittent injections of sodium pentobarbital throughout the course of the study. Cannulae were placed in the femoral artery and femoral vein and a catheter was inserted for urine collection. PALA (120 mg/kg containing 63.67 microcuries of ^{14}C), dissolved in H_2O, was infused over 2 minutes via the iv cannula. Serial arterial blood samples were obtained and analyzed by liquid scintillation counting for total radioactivity and by the GC method and SIM for PALA equivalents. The ^{14}C content in 0.2-ml aliquots of serum was determined by adding 1 ml of NCS tissue solubilizer (Amersham/Searle Corp, Arlington Heights, Ill) and then incubating for 1 hour at 48°C. Eighteen ml of the scintillation solution was added and the samples were analyzed on a MARK III Model 66880 Liquid Scintillation System equipped with automatic quench correction (Searle Analytical Inc, Des Plaines, Ill).

Isolation and Derivatization Procedures

Aliquots (0.5 ml) of serum were placed in 12 × 75-mm disposable tissue culture tubes. Samples containing PALA at a concentration > 15 μg/ml were diluted with bovine serum before analysis. Fifty μl of the internal standard solution was added and the solution was thoroughly mixed using a vortexer. Precipitation of the protein was achieved by adding 2 ml of acetone followed by thorough mixing with a vortexer, heating at 55°C–60°C for 10 minutes, and then sonicating for 5 minutes in an ultrasonic cleaner, Model 4.6 (Mettler Electronics, Anaheim, Calif). After centrifuging for 2 minutes at 800 × *g* the acetone layer was discarded. Two ml of acetone was added to the residue and the above procedure was repeated except that the heating step was omitted. In order to remove the remaining water in the serum, 2 ml of diethyl ether was added to the residue. The contents were mixed using a vortexer, sonicated for 5 minutes, and centrifuged, and the serum was dried at 55°C–60°C.

Extraction of PALA and HOMOPALA from the dried serum was accomplished in the following manner: 1 ml of tetrahydrofuran and 200 μl of methanolic/HCl (1.5 M) were added to the serum. The contents were thoroughly mixed using a vortexer, sonicated for 5 minutes, and centrifuged for 2 minutes at 800 × *g*. The supernatant was transferred to a clean 12 × 75-mm disposable tissue culture tube. The residue was extracted a second time and the two extracts were combined and evaporated at room temperature under a stream of dry nitrogen. The tetramethyl derivatives were prepared by adding 0.3 ml of chloroform and 10 μl of methanolic/HCl (3.0 M) to the serum extract. Diazomethane in chloroform was then added until the solution remained yellow (approximately 1.0–1.6 ml). Because the diazomethane in chloroform was not stable when stored, it was used immediately following its preparation. The solution was sonicated for 1–2 minutes and evaporated under a stream of dry nitrogen. The derivatized compounds (fig 1) were dissolved in 100 μl of benzene and 2.0- and 3.0-μl aliquots were injected into the gas chromatograph or gas chromatograph/mass spectrometer for analysis respectively. Analysis of PALA in urine was the same as for serum except that 0.2-ml aliquots were analyzed and 200 μl of the internal standard (180 μg/ml in H_2O) was added to the sample. In addition, samples containing PALA at a concentration > 200 μg/ml were diluted with water before analysis.

Instrumentation

Quantitation by the GC method was performed on a Hewlett Packard Model 5710A Gas Chromatograph equipped with a dual nitrogen-phosphorus flame ionization detector (Hewlett Packard, Avondale, Pa). Chromatographic separation of PALA and HOMOPALA was achieved using 2 mm (internal diameter) × 1.3-m long glass column packed with 80/100 mesh 3% OV-210 on Chromosorb W-HP (Pierce Chemical Co, Rockford, Ill). The flow of helium carrier gas was 30 ml/minute. The injector port and detector temperatures were 250°C and the column temperature was 180°C.

Qualitative identification of PALA and HOMOPALA

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[6] Pharmacology and radioautography of antitumor agents. Contractor's report to the Division of Cancer Treatment, National Cancer Institute, from Arthur D. Little, Inc, Cambridge, Mass (contracts N01-CM-53849 and N01-CM-87163), Apr 16, 1977, to July 1, 1978, pp 70–71.

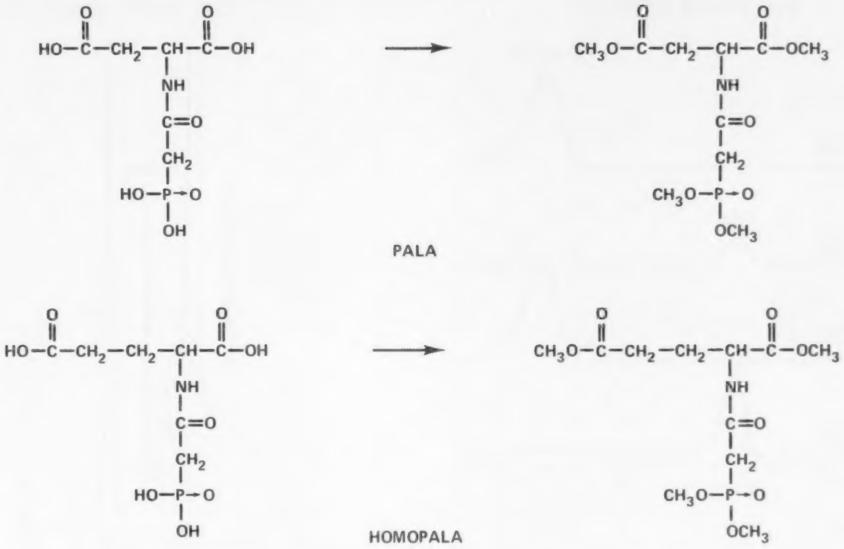

FIGURE 1.—Structural formulas of PALA, HOMOPALA, and their tetramethyl derivatives.

and quantitation of PALA by SIM were accomplished using a Hewlett Packard Gas Chromatograph Mass Spectrometer Model 5980A equipped with a 5933A Data System. The ionizing energy was 70 ev and the transfer line was maintained at 250°C. GC parameters were as described.

Quantitation

Serum samples (0.5 ml) containing 0.5, 0.7, 1.0, 3.0, 5.0, 7.0, 10.0, and 15.0 μg/ml of PALA and urine samples (0.2 ml) containing 20.0, 50.0, 100.0, 150.0, and 200.0 μg/ml of PALA were analyzed using the procedure described above. Quantitation of PALA by the GC analysis was achieved by determining the peak height ratio of the drug to internal standard. For analysis by SIM a recording was made of the ion intensities at m/z 220 and m/z 252 (PALA) and m/z 234 and m/z 266 (HOMOPALA). Loss of O-$COCH_3$ from the PALA and HOMOPALA molecular ions most likely produced the ions at m/z 252 and m/z 266 respectively. An additional loss of CH_3OH would then give rise to the ions at m/z 220 and m/z 234. The ratio of the two ion intensities in each compound was used to confirm their identity and quantitation was based on the ion intensity ratios of m/z 220 (PALA) and m/z 234 (HOMOPALA). Standard curves were constructed to determine the linearity over the range of the analysis for both the GC and SIM methods. At least seven replicate samples of the highest and lowest serum and urine standard solutions were analyzed to determine the precision of the method. Overall recovery of PALA from the serum and urine was determined by analyzing reference serum samples treated with PALA-^{14}C. Both the acetone and ether washes were measured for radioactivity as well as the extract.

RESULTS AND DISCUSSION

Analysis of PALA

PALA and HOMOPALA were identified in extracts of serum and urine by electron impact gas chromatography/mass spectrometry and compared with those obtained for the reference compounds. The mass spectrum of PALA was also in good agreement with that previously reported (5). Using our derivatization procedure the pentamethyl derivatives reported by the same authors for PALA were always less than a few percent of the tetramethyl derivatives for both PALA and HOMOPALA and in most cases were below our limits of detection.

The standard curve constructed from data obtained by analysis of the reference standard PALA solutions was linear over the range 0.5–15.0 μg/ml (serum) and 20–200 μg/ml (urine) for both the GC and SIM methods. Multiple extractions of the highest and lowest serum standards gave good reproducibility, ie, 15.0 μg/ml ± 7.5% (SD) and 0.5 μg/ml ± 2.6% (SD) when analyzed by GC and 15.0 μg/ml ± 11.0% (SD) and 0.5 μg/ml ± 9.0% (SD) when analyzed by SIM. The results of the analysis of multiple urine standards by SIM were 20 μg/ml ± 9.5% (SD) and 200 μg/ml ± 7.8% (SD).

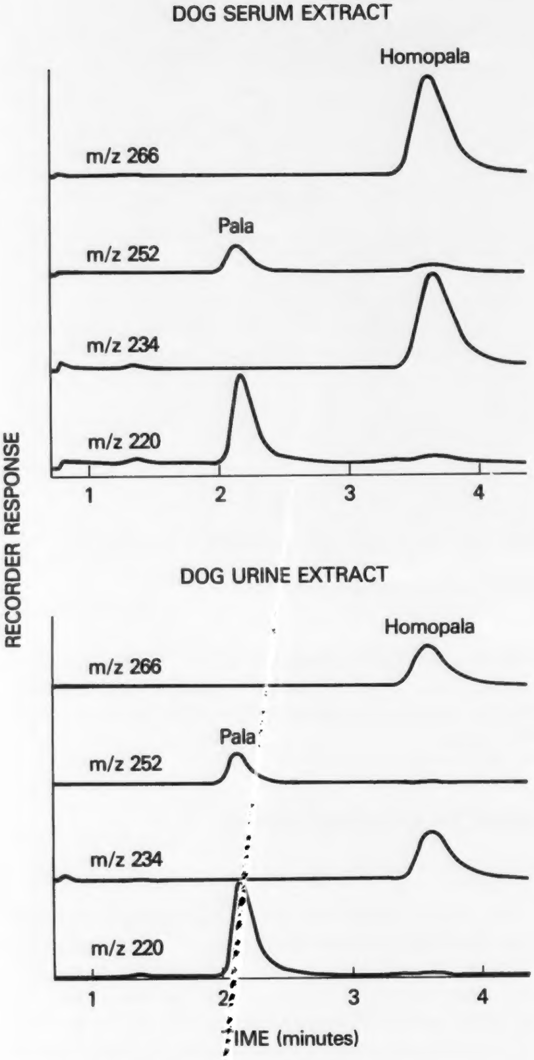

FIGURE 2.—*a*. SIM tracing from a dog serum extract. The sample was extracted from 0.5 ml of serum containing 4.5 μg/ml of PALA and 18 μg/ml of HOMOPALA and represents 135 ng of PALA and 210 ng of HOMOPALA injected on column. *b*. SIM tracing from a dog urine extract. The sample was extracted from 0.2 ml of urine containing 132 μg/ml of PALA and 72 μg/ml of HOMOPALA and represents 396 ng of PALA and 1 μg of HOMOPALA injected on column.

Overall extraction efficiency of PALA determined with PALA-^{14}C averaged 77.7% ± 18.2% (SD) for four separate determinations. Loss in the acetone and ether wash solutions in no case was > 1.5%. Stability of the tetramethyl derivatives was assessed and they were found to be stable in the final benzene solutions for at least 9 days.

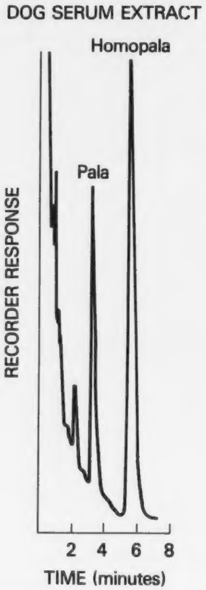

FIGURE 3.—GC tracing of a dog serum extract. The sample was the same as shown in fig 2a.

However, an attempt to extract the derivatives from water into an organic solvent as a final cleanup procedure before analysis resulted in decomposition, possibly as a result of hydrolysis of the phosphonic acid ester groups. Typical tracings from a dog's serum and urine extract containing PALA and the internal standard HOMOPALA are shown in figures 2a and 2b respectively. Figure 3 is the same dog's serum extract as shown in figure 2a but analyzed by the GC method. No interferences were observed in control sera or urine when using SIM. However, control serum samples which had undergone hemolysis contained an unidentified compound which eluted with the same GC retention time as PALA. Analysis of whole blood indicated that this compound was contained in the cellular fraction.

Although PALA is highly water-soluble, a lengthy cleanup procedure was not required for quantitation since the methods of detection were highly specific. Initial attempts were made to remove proteins and lipids from the serum using a modification of the method reported by Roboz et al (6) for analysis of phosphonacetic acid. This method used $HClO_4$ to deproteinize the serum followed by extraction with dichloromethane to remove the lipids. However, we found that PALA was not stable under the conditions required for protein removal. It was also noted, as reported by Roboz et al, that drying the serum by lyophilization was not successful. Therefore, we used acetone washes followed by an ether wash to azeotrope

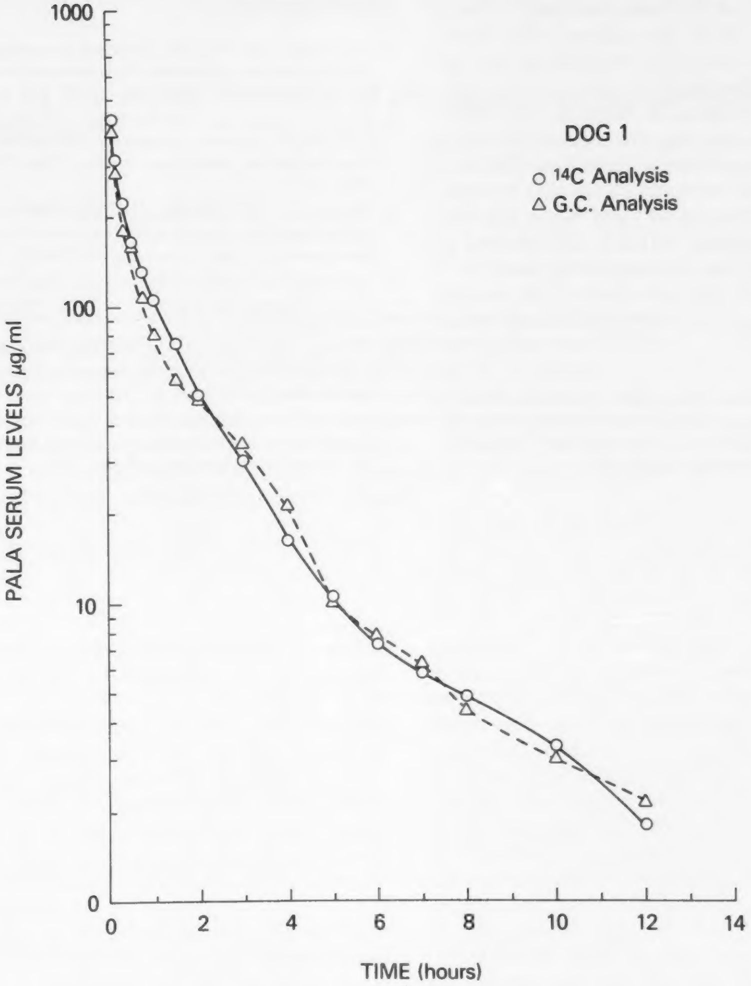

FIGURE 4.—Dog serum PALA levels (△) and total radioactivity (○) with time after a 120-mg/kg iv dose of PALA containing 63.67 microcuries of PALA-^{14}C.

the water from the serum prior to extraction with an anhydrous HCl/tetrahydrofuran/methanol solution.

Animal Studies

Serial arterial blood samples were analyzed for PALA by the GC method and for total radioactivity following iv administration of PALA-^{14}C in the dog (fig 4). In general, the ^{14}C content paralleled that of PALA equivalents determined by the GC technique. Peak serum levels in the two dogs averaged 447 µg/ml (by radioactivity determination) and 425 µg/ml of PALA equivalents (by GC analysis). The absolute mean differences between total radioactivity and PALA equivalents determined in the serum samples were 12.6% (dog 1) and 14.2% (dog 2) and the total radioactivity accounted for as PALA was 97% and 90% respectively. Total radioactivity accounted for in the urine 12 hours after infusion of PALA averaged 82.0% of the dose for the two dogs. PALA equivalents measured as total radioactivity and by SIM were obtained for the first 3-hour urine collections and the first 8-hour urine collections in dogs 1 and 2 respectively. A comparison of these data in dog 1 showed that 62.7% of the dose was recovered in the urine as determined by total radioactivity and 67.7% of the dose was accounted for as PALA using the SIM method. Similarly, for dog 2 the values were 84.2% and 83.4% of the dose recovered in the 8-hour urine collections. These data support other observations that PALA is primarily excreted as the unchanged compound in the dog.[7]

Further work is in progress to define the pharmacokinetics of PALA in man and dog. The methods described are being used in these studies to quantitate PALA in urine and serum but the methods should also be applicable to cerebrospinal fluid (CSF) and tissue extracts. Initial attempts to quantitate PALA in CSF showed no interferences; however, the maximum levels detected in the dog studies were at the lower level of the analysis and therefore no attempt was made to quantitate PALA in the CSF samples.

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[7] Pharmacology and tumor bank. Final report. Contractor's report to the Division of Cancer Treatment, National Cancer Institute, from Arthur D. Little, Inc, Cambridge, Mass (contract N01-CM-53849), Mar 1, 1975, to Apr 15, 1977, vol 2 (Pharmacology), pp 463–489.

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Reduction of the Renal Toxicity of *cis*-Dichlorodiammineplatinum(II) by Probenecid[1,2]

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SUMMARY

Probenecid, when administered sc to F344 rats 1 hour prior to an iv LD50 dose of *cis*-dichlorodiammineplatinum(II) (CDDP), reduced significantly the peak BUN and serum creatinine levels which occurred 4 days following CDDP. Renal histopathology was similarly reduced by probenecid pretreatment. In BDF_1 mice, the LD50 value of CDDP administered ip without probenecid pretreatment was 17 mg/kg; the LD50 dose with probenecid was increased to $>$ 19 mg/kg. Probenecid pretreatment did not influence the therapeutic response to CDDP when the latter was administered to BDF_1 mice bearing L1210 leukemia.

[Cancer Treat Rep 63:781–787, 1979]

cis-Dichlorodiammineplatinum(II) (CDDP) is a unique antitumor agent which shows significant activity in man against a number of tumors (1–4). Its dose-limiting nephrotoxicity was recognized early in clinical trials and has been thoroughly documented (5–9). In 1976 it was observed that renal toxicity could be ameliorated by hydration and diuresis concomitantly with CDDP administration (10,11), and consequently, prehydration with or without diuretics has been employed extensively in the administration of CDDP (*see* refs 12–14 as representative examples).

In a comparison of the relative renal toxic effects of some organoplatinum analogs, Ward et al (15) developed an animal model using F344 rats and measured serum creatinine and BUN levels at intervals after a single iv injection of CDDP at the LD50 level. With CDDP, a proximal tubular degeneration period of 2 days occurred during which no significantly elevated creatinine or BUN values were noted. During a necrosis period (Days 2–5), both values peaked, with the maxima occurring at 4 days. A regeneration period (Days 5–14) was characterized by a return of creatinine levels to virtually normal values; BUN levels declined rapidly between Days 4 and 7 and then declined more slowly and were still well above normal values on Day 14. Ward and co-workers subsequently showed that furosemide protects against the renal toxicity induced by a single injection (16) or weekly injections (17) of CDDP. These studies were extended by Pera and Harder (18), who concluded that the renal protection afforded by furosemide or mannitol was due primarily to resultant lower concentrations of platinum (Pt) in the urine. Kidney Pt content in rats receiving mannitol was similar to that of rats receiving CDDP alone, but furosemide-treated animals actually had higher kidney Pt levels at 24 hours and 4 days following CDDP administration. Pera and Harder's preliminary studies suggested that mannitol, unlike furosemide, may protect against the lethality caused by high doses of CDDP.

In spite of the extensive clinical use of hydration with or without diuresis to reduce the renal toxicity of CDDP, this maneuver is not without disadvantages. Hospitalization is usually required, and additional supportive nursing care is necessary; thus, additional costs are incurred. Considerations other than economic ones are also of some importance. Hayes et al (10) reported severe electrolyte disturbances in two of 75 treatment courses which were

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[2] Supported by the Medical Research Service of the Veterans' Administration, and by Public Health Service grants CA-11811 (National Cancer Institute), GM-07404 (National Institute of General Medical Sciences), and RR-05426 (Division of Research Resources) from the National Institutes of Health, Department of Health, Education, and Welfare.

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[5] Alayne B. Smith, Loretta M. Atkins, and Charlotte S. Reed provided skilled technical assistance. Drs. Francis M. Brown (Laboratory Service, Veterans' Administration Medical Center) and Ernest M. Walker, Jr (Department of Laboratory Medicine, Medical University of South Carolina) independently evaluated the histopathology of renal sections. Jon R. Benthal (Medical Media Production Service, Veterans' Administration Medical Center) prepared the photomicrographs.

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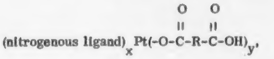
attributed to hydration and diuresis. It is apparent that a simpler therapeutic intervention to reduce the renal toxicity of CDDP could have some advantages over those employed currently, and this consideration prompted us to investigate a possible alternative.

Morris and Gale (19) synthesized a ^{3}H-labeled bis-pyridine analog (PPC) of CDDP and investigated its binding to a variety of biologic molecules using an equilibrium dialysis technique. They also observed the ways in which various amino acids influenced the dissociation of the labile chlorides which are common to both PPC and CDDP. On the basis of those data, it seems likely that in a complex in vivo environment the chlorides of CDDP may be displaced by certain molecules which, after binding with the Pt, would yield a compound(s) containing a free carboxyl group. A simple example would be:



$$(\text{nitrogenous ligand})_x\text{Pt}(\text{-O-}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{-R-}\overset{\overset{\text{O}}{\|}}{\text{C}}\text{-OH})_y,$$

where x = 2 in the case of the monodentate ammine or pyridine ligand and y = 2 for a dicarboxylic acid in which R = > 3 carbon atoms. Such a reaction would thus make it a candidate molecule for active transport in the kidney by the *p*-aminohippurate (PAH) carrier system, and this system is known to be inhibited by probenecid (20,21). Also pertinent to this hypothesis are the data of Child and Dodds (22) and Tune (23,24) which showed that the renal accumulation and consequent toxicity of cephaloridine can be prevented by the prior administration of probenecid.

The current preliminary study was initiated to determine if probenecid influences the renal toxicity which follows the administration of CDDP.

MATERIALS AND METHODS

CDDP was synthesized by the method of Kaufman and Cowan (25) and was dissolved in 0.9% NaCl immediately prior to use. Probenecid [*p*-(dipropylsulfamyl)benzoic acid] was obtained from the RSA Corp (Ardsley, NY). It was dissolved in 1.0 N NaOH, titrated to pH 7.4 with 1.0 M KH_2PO_4, and the resulting clear solution was diluted to the desired volume with 0.9% NaCl (26). An identical vehicle solution devoid of probenecid was prepared for appropriate control injections. The (C57BL/6 × DBA/2)F_1 hybrid male mice (BDF$_1$) and F344 male rats were furnished by the Mammalian Genetics and Animal Production Section, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute (Bethesda, Md). The rats were obtained at 4 weeks of age and were quarantined 2 weeks prior to use to assure that they were free of mycoplasma infection. Reagents for BUN and creatinine determinations were obtained as the Rapid Stat kits from Pierce Chemical Co (Rockford, Ill).

In the experiments to assess the renal toxicity of CDDP in rats, the protocol was virtually identical to that described by Ward et al (15). Groups of five rats each received CDDP at a dose of 7.4 mg/kg iv, probenecid at a dose of 100 mg/kg sc, a combination of the two, or the appropriate solvent control solutions. In all instances, probenecid was administered 1 hour prior to CDDP injection. At the desired times after treatment, each rat was weighed and anesthetized with ether, blood for BUN and creatinine determinations was obtained by cardiac puncture, a bilateral nephrectomy was performed, and the kidneys were stripped of connective tissue, decapsulated, quickly weighed, bisected longitudinally, and placed into Tellyesniczky's fixative (37%–40% formaldehyde:glacial acetic acid:70% ethyl alcohol in the ratio 5:5:100). After embedding, the sections were stained by the alcian blue-periodic acid-Schiff method and with hematoxylin-eosin.

For acute toxicity tests, BDF$_1$ mice were divided into groups of ten and were injected ip with CDDP 1 hour after sc injection of probenecid or the control probenecid solvent solution. The observation period was 21 days, during which survivors were recorded daily.

To determine if probenecid influences the therapeutic response to CDDP, groups of ten BDF$_1$ mice were injected ip with 10^6 L1210 leukemia cells. After 23 hours, mice received either the probenecid solution or the probenecid control solvent sc. This was followed after 1 hour by a single ip injection of CDDP using a wide range of sublethal doses. Survivors were recorded twice daily. Observations were terminated at 60 days, and mice surviving at that time were recorded as "cured" but were entered into calculations of survival times as having survived only 60 days (1440 hours).

Statistical analyses of data were done with a Wang programmable calculator using a cassette-taped program of Student's t test prepared in the Department of Biometry, Medical University of South Carolina, or by the chi-square test, as appropriate.

RESULTS

BUN and serum creatinine values obtained on Day 2 from all treated groups were not different from the values of the control group. This was altogether concordant with the data obtained by Ward et al (15). On Day 3, however, BUN values began to increase. Rats treated with only CDDP had a mean BUN value of 56.9 ± 18.5 mg/dl (control value = 18.1 ± 2.0 mg/dl), and the mean value obtained from the group treated with probenecid prior to CDDP was 62.2 ± 23.4 mg/dl, indicating no effect of probenecid on Day 3. Day-3 values of serum creatinine were virtually the same in all groups.

Table 1 shows results of the Day-4 evaluation. Rats treated with only probenecid had BUN and creatinine levels which did not differ significantly from those of

TABLE 1.—Effects of probenecid on the elevation of BUN, serum creatinine, and kidney:body weight ratio of F344 rats when measured 4 days after iv injection of an LD50 dose of CDDP*

Treatment	Serum values: BUN (mg/dl ± 1.0 SD)	Serum values: Creatinine (mg/dl ± 1.0 SD)	Kidney/body weight × 100 ± 1.0 SD
Solvents only	21.1 ± 2.3	1.17 ± 0.15	0.75 ± 0.02
100 mg/kg of probenecid	17.6 ± 1.7	1.10 ± 0.35	0.76 ± 0.04
7.4 mg/kg of CDDP	162.6 ± 39.4	5.17 ± 0.77	1.03 ± 0.15
Probenecid + CDDP	60.5 ± 11.2	3.30 ± 0.67	0.81 ± 0.07

*Rats received probenecid (or the probenecid solvent) sc 1 hr before CDDP treatment.

solvent-treated controls. The rats that received only CDDP had BUN and creatinine levels which were about 6.7- and 3.4-fold higher than control values, respectively. These increases were highly significant ($P < 0.001$). With probenecid pretreatment, the elevated BUN value was only about 1.9-fold higher than the control value; the difference between the groups receiving CDDP with and without probenecid was highly significant ($P < 0.005$). Probenecid similarly reduced the CDDP-induced increase in serum creatinine to about 1.8-fold the control value, and comparison of the CDDP group and the probenecid plus CDDP group revealed a high degree of significance of the difference ($P < 0.01$). Inspection of the column in which the kidney:body weight ratios are recorded reveals a moderate increase (37%; $P < 0.025$) caused by CDDP treatment. Admittedly, the pathophysiologic basis of this is obscure, since it no doubt reflects some actual elevation of renal weight (17) coupled with loss of total body weight due to toxicity. The degree of amelioration of this increased ratio by the prior administration of probenecid eluded significance at the 95% confidence level ($P \sim 0.07$) due to the large standard deviation of the mean ratio of the CDDP group.

Since we used essentially the same protocol as described by Ward et al (15) and thus injected CDDP at its LD50 dose in this rat strain, death occurred after 4 days and rendered statistical treatment of the subsequent data substantially invalid. However, mere inspection of the limited data on BUN and creatinine values obtained from the few animals remaining alive after 4 days showed a pattern similar, through Day 6, to that shown in table 1.

Figure 1 shows longitudinal hemisections of representative kidneys from rats that received control solvents only, probenecid only (97 hours before being killed), CDDP only (96 hours before being killed), and probenecid plus CDDP. Note that the acetic acid in the fixative produced marked deposits of acid hematin in the blood engorging the medullary areas in kidneys from rats that received CDDP alone, indicating progression of the organ toward failure. This was not evident after fixation of any of the kidneys from rats that received probenecid before CDDP.

Figure 2 shows a representative field in the cortical area of a solvent-control rat and reveals normal histology.

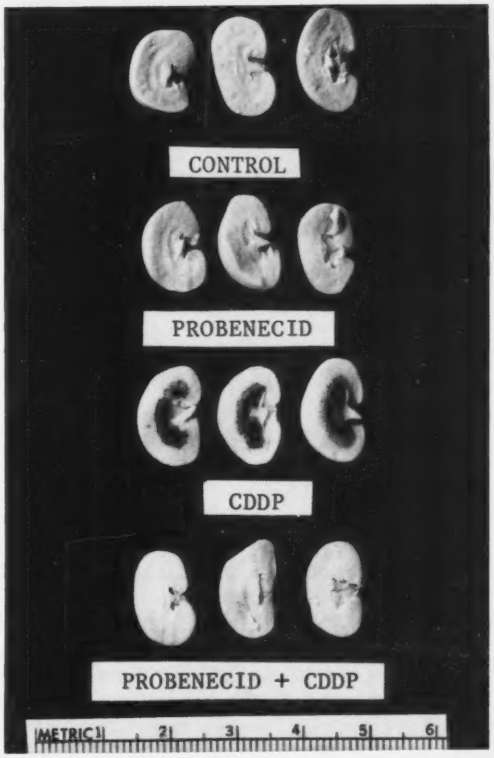


FIGURE 1.—Longitudinal hemisections of representative kidneys from rats treated as indicated. CDDP at a dose of 7.4 mg/kg iv was administered 96 hrs before rats were killed. Probenecid at a dose of 100 mg/kg sc was injected 1 hr prior to CDDP administration. Note the fixative-induced acid hematin in the medullary areas of kidneys from rats treated with CDDP alone and its absence in the kidneys from rats that received probenecid prior to CDDP.

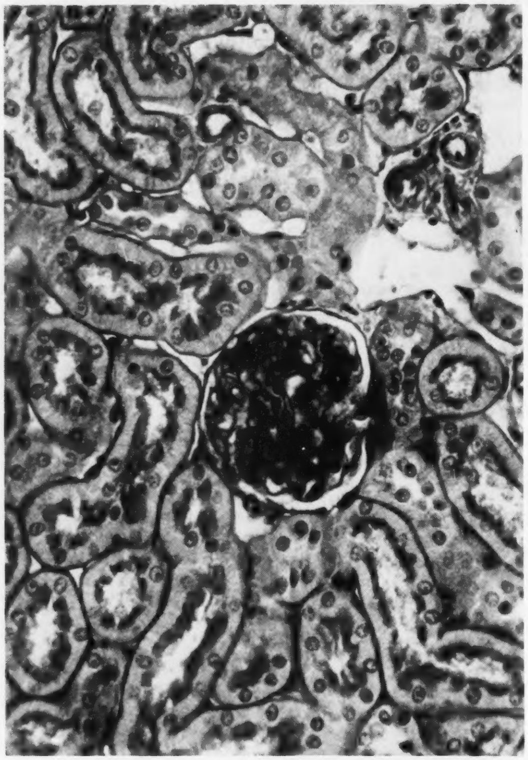


FIGURE 2.—Section from cortical area of control rat kidney. Tellyesniczky's fixative, alcian blue-periodic acid-Schiff stain, × 300. This field reveals normal morphology with no obvious pathology. Brush borders are prominent and normal endothelial detail is evident.

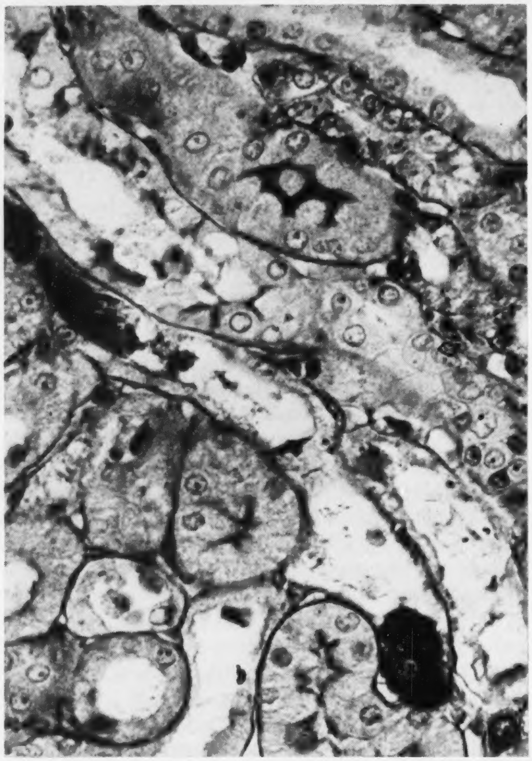


FIGURE 3.—Same as fig 2, except the rat received 7.4 mg/kg of CDDP iv 96 hrs before being killed. Note loss of brush border, extensive destruction and desquamation of epithelium, and presence of intraluminal casts.

A corresponding photomicrograph of tissue from a rat that received only probenecid is not shown, since none of these could be distinguished from control sections.

A section from a rat that received CDDP 96 hours before being killed is shown in figure 3. Glomeruli (not shown) were substantially normal. However, there were pronounced changes in the proximal tubules which included (*a*) almost complete loss of brush borders, (*b*) extensive epithelial changes including coagulation necrosis, (*c*) destruction and desquamation of necrotic epithelium into lumina (in most fields this material was still partially attached to underlying basement membranes, but sometimes completely detached), and (*d*) filling of lumina with eosinophilic granular cytoplasmic debris. Eosinophilic, periodic acid-Schiff-positive granules were observed frequently within the cytoplasm of intact cells, possibly representing attempted resorption of excess protein from the lumina. Distal tubules were relatively intact with no remarkable necrotic changes. Casts were observed within the nephrons and were most pronounced as broad casts within collecting ducts seen in renal pyramids.

Sections from rats that received probenecid 1 hour before CDDP (fig 4) revealed some of the changes seen in the sections from rats that received only CDDP, but all were less extensive. Brush borders were relatively intact. Evidence of damage to proximal tubular epithelium was apparent with some sites of cellular necrosis, but there was minimal obvious destruction and desquamation of tubular epithelium into lumina. Casts were present, most pronounced in collecting ducts, but were much fewer in number and caused less architectural disruption of renal pyramids than those observed in the group that received CDDP alone.

The effect of probenecid pretreatment on the acute toxicity of CDDP in BDF_1 mice is shown in table 2. With CDDP only, the LD50 value was 17 mg/kg; when probenecid was given 1 hour before CDDP, the correspond-

TABLE 2.—Effect of probenecid on the acute toxicity of CDDP in BDF_1 mice*

CDDP (mg/kg)	Probenecid (mg/kg)	Cumulative mortality/10 mice on Day—					
		5	6	7	8	9	21
17	0	0	0	4	5	5	5
18	0	0	2	7	7	7	7
19	0	0	2	7	8	8	8
17	100	0	0	0	0	0	0
18	100	0	0	0	0	0	0
19	100	0	1	1	2	2	2

*Mice received probenecid (or the probenecid solvent) sc 1 hr before CDDP treatment.

ing value was > 19 mg/kg. This difference, even though modest, was highly significant ($P < 0.01$).

Table 3 shows that pretreatment with probenecid did not apparently influence in any way the therapeutic action of CDDP on L1210 leukemia in BDF_1 mice. Statistical comparisons of groups at the same dose level did indeed show that a significant difference ($P < 0.05$, > 0.025) was evident at 12 mg/kg. However, this was not true at ≤ 10 mg/kg or at 14 mg/kg. Even though the difference at 12 mg/kg was significant from the *statistical* standpoint (obviously due to the fact that two 60-day survivors were included in one group used in the computations), the *practical* significance of the difference is probably not important.

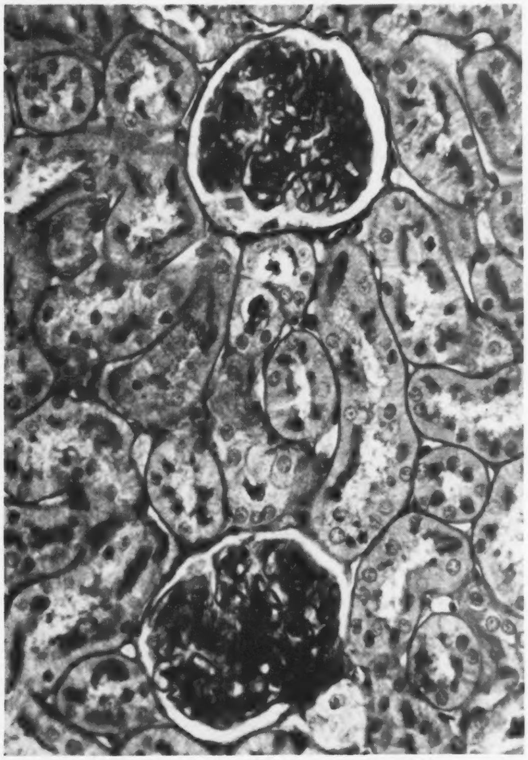


FIGURE 4.—Same as fig 3, except the rat received 100 mg/kg of probenecid sc 1 hr before administration of CDDP. Brush borders are relatively intact, most nuclei appear substantially normal, and only slight degeneration of epithelium can be detected.

DISCUSSION

The data presented indicate that prior administration of probenecid reduces the renal toxicity of CDDP in rats as assessed by histopathologic evaluation and by measurements of BUN and serum creatinine levels. In addition, prior treatment with this inhibitor of the PAH transport system increases the dose of CDDP required to kill 50% of a mouse population, while not influencing the therapeutic action of CDDP in a mouse leukemia model. The mechanism by which such a protective action is expressed is not at all clear at this time.

The renal excretion kinetics of CDDP have not been explored in depth. Litterst et al (27) measured plasma

TABLE 3.—Effects of probenecid on survival time of BDF_1 mice bearing L1210 leukemia that were treated with CDDP*

CDDP (mg/kg)	Mean survival time (hrs) ± 1.0 SD†	
	No probenecid	Probenecid (100 mg/kg)
0	187 ± 22	202 ± 24
1	262 ± 15	272 ± 35
2	301 ± 33	327 ± 46
4	588 ± 300 (1)	424 ± 97
6	534 ± 309 (1)	507 ± 101
8	571 ± 129	563 ± 182
10	628 ± 281 (1)	884 ± 391 (3)
12	736 ± 405 (2)	404 ± 127‡
14	764 ± 463 (3)	533 ± 363 (1)

*Groups of 10 mice received 10^6 L1210 cells on Day 0. Treatment with CDDP was on Day 1 only. Mice received probenecid (or the probenecid solvent) sc 1 hr prior to CDDP treatment.

†Nos. in parentheses = No. of cures (> 60-day survivors) in each group of 10 mice.

‡$P < 0.05$, > 0.025.

clearance and organ distribution in beagle dogs following a single iv injection and found a biphasic clearance pattern (half-life $<$ 1 hour and $\sim$ 5 days). Initial concentrations of Pt were highest in excretory organs, gonads, spleen, and adrenals, but remained elevated for prolonged periods only in liver, ovary, uterus, and kidney. Their protocol was not designed to detect any role of tubular secretion in the excretory process. Slater et al (28) attempted to modify rat nephrotoxicity of CDDP by administration of thiol reagents. Penicillamine, cysteamine, or acetylcysteine given to rats after CDDP was in no way protective. Those authors concluded that the binding of Pt to proximal tubular membranes was of unusual tenacity, but they presented no data to indicate whether excretion was by glomerular filtration, tubular secretion, or both.

The data of Ward et al (15–17) suggest that the protective effects of furosemide and mannitol on CDDP-induced nephrotoxicity may not be due exclusively to their diuretic actions. In one report, they listed other actions of furosemide (effect on sodium and fluid transport; inhibition of cellular metabolic processes, binding to plasma proteins) and of mannitol (unspecified effect on proximal tubules) to support such a contention (16). Even though Ward and co-workers (16,17) documented a protective action of furosemide, Pera and Harder (18) actually found higher Pt levels in the kidneys of furosemide-treated rats which then received CDDP than in the kidneys from rats which received CDDP alone.

Tune (24) compared the renal transport of PAH and cephaloridine, and offered an explanation of the protective action of probenecid on cephaloridine nephrotoxicity. PAH is transported by filtration and to a greater extent by secretion. Cortical PAH is composed of large intracellular and intraluminal components; thus, medullary PAH includes a filtered fraction and a larger secreted fraction. Cortical and medullary components are reduced by probenecid. Cephaloridine is excreted principally by glomerular filtration. In addition, it is actively transported across the peritubular membrane into the proximal tubular cell by the PAH carrier. However, its subsequent movement across the luminal cell membrane is in some way restricted, resulting in inordinately high intracellular concentrations. Probenecid blocks its peritubular transport and thus prevents attainment of high intracellular levels. It would be tempting, but perhaps premature, to speculate that one or more adducts of CDDP, formed in vivo after chloride dissociation, may be transported in a manner analogous to that of cephaloridine, and it is this species which is predominantly responsible for renal toxicity. Such a suggestion would be concordant with the observation of Pera and Harder that furosemide-"protected" kidneys of rats actually contained higher concentrations of Pt after CDDP administration than the kidneys of rats treated with CDDP only (18).

The clinical utilization of CDDP is increasing at a rapid rate. At the present time, no analog with clearly reduced renal toxicity is available. It is hoped that the observations reported here will be extended by other investigators to define the possible role of probenecid as an adjunct to CDDP administration, but with the awareness that any altered distribution pattern which may be induced could influence the spectrum of antitumor action of the drug.

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Phase II Study of Anguidine in Advanced Breast Cancer[1,2]

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SUMMARY

A phase II evaluation of anguidine was carried out in 30 patients with advanced refractory breast cancer. A dose of 5.0 mg/m^2 daily for 5 days was explored. The main toxic effects were nausea and vomiting, fever and chills, hypotension, skin erythema, somnolence, confusion, and lethargy. Myelosuppression was minimal. Among these extensively pretreated patients, there was one partial responder and one additional patient who showed improvement (less than a partial response); both responses occurred in soft tissue sites.

[Cancer Treat Rep 63:789–791, 1979]

Despite recent advances in the treatment of advanced metastatic breast cancer, durable responses are rarely obtained (1–4). There is, therefore, a need for a vigorous search for new antitumor agents to be utilized in the event of primary multiple-drug failure. The aim of the present study was to evaluate the potential antitumor activity of anguidine in patients with advanced refractory metastatic breast cancer.

Anguidine is one of a new class of agents derived from fungi. It is the principal phytotoxic metabolic product of the plant parasitic fungus *Fusarium equiseti* but has also been isolated from culture filtrates of *F. diversiporum*, *F. sambucinum*, *F. scirpi*, and *Gibberella intricans*. It was selected for phase I clinical studies based on its activity in the P388 tumor system, in which it induced a reproducible increased lifespan of > 100% when given as an ip injection in a repeated-dose schedule (daily for 9 days).[5] In a phase I study of anguidine conducted at the M. D. Anderson Hospital (5), a dose of 5.0 mg/m^2 daily for 5 days gave maximum tolerable side effects in the majority of patients; this dose was selected for the present study. However, in the phase I study, patients with hepatic metastases and/or liver enzyme elevations > 50% experienced excessive toxicity. Thus, a dose of 3.0 mg/m^2 daily for 5 days was selected for patients with abnormal findings on liver scan and/or liver enzyme abnormalities (> 50% elevation).

MATERIALS AND METHODS

Thirty patients with histologically proven breast cancer refractory to more conventional chemotherapeutic agents including Adriamycin, 5-fluorouracil, cyclophosphamide, and methotrexate were entered in this study. The starting dose schedule for all patients with no abnormal findings on liver scans and normal liver enzyme levels (≤ 50% elevation) was 5.0 mg/m^2 daily for 5 days, to be repeated every 3 weeks. Patients with abnormal findings on liver scans and/or liver enzyme abnormalities (> 50% elevation) were treated initially with a dose of 3.0 mg/m^2 daily for 5 days, repeated every 3 weeks. All patients treated had evidence of measurable disease and all gave written informed consent in accordance with our institutional policy.

Partial remission was defined as a > 50% reduction in the size of measurable lesions. Improvement was defined as a < 50% reduction in the size of measurable lesions. Stable disease was defined as a steady state of response (less than improvement) or progression (less than increasing disease); there could be no new lesions for this category. Increasing disease was defined as an unequiv-

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[3] Medical Breast Service, Department of Medicine (*H.-Y. Yap, A. DiStefano*, and *G. R. Blumenschein*), and Department of Developmental Therapeutics (*W. K. Murphy* and *G. P. Bodey*), The University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, Houston.

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TABLE 1.—Pretreatment patient characteristics

Age (yrs)	
Median	52
Range	30–74
Disease-free interval (mos)	
Median	15
Range	0–42
Duration of prior therapy (mos)	
Hormonal therapy	
Median	4
Range	1.5–57
Chemotherapy	
Median	16
Range	5–37
Total duration of prior therapy	
Median	22
Range	9–62
Performance status (Karnofsky)	
Median	7
Range	5–10

TABLE 2.—Hematologic toxicity in 36 evaluable courses

	Cells × 10^3/mm^3	
	Median	Range
Lowest total wbc count	3.6	2.0–8.7
Lowest absolute granulocyte count	2.2	0.8–6.8
Lowest platelet count	150	12–511

TABLE 3.—Nonhematologic toxic effects in 52 evaluable courses

Symptoms	% of courses
Nausea + vomiting	87
Fever ± chills	33
Hypotension	
Symptomatic	7
Asymptomatic	24
Skin erythema	22
Burning sensation	4
Central nervous system effects	
Somnolence	22
Confusion	6
Lethargy	10
Hair loss	4

ocal increase of at least 25% in the size of any measured lesion. Appearance of significant new lesions was also considered to constitute increasing disease.

Of the 30 patients entered in this study, five were not evaluated for response because of inadequate trial. None of these five patients completed their first course of treatment due to excessive toxicity. They were, however, evaluated for toxicity.

RESULTS

Among the 25 evaluable patients, one patient achieved a partial response with almost complete disappearance of soft tissue disease and stability of diffuse osseous metastases. Unfortunately, she died of sepsis while still in remission 11 weeks from the start of therapy. An additional patient had less than partial response of her chest wall lesions, which lasted for 12 weeks. Ten patients achieved stability of their disease for 9–20+ weeks (median, 15 weeks). Increasing disease was evident in 13 patients. Table 1 shows the pertinent pretreatment patient characteristics. All had extensive prior therapy with a median duration of 22 months (range, 9–62 months). The hematologic and nonhematologic toxic effects are listed in tables 2 and 3 respectively. The most significant side effect was hypotension. In three patients, symptomatic hypotension occurred within 12–24 hours after the first dose of anguidine. All three patients required aggressive antihypotensive therapy, including iv infusion of large volumes of normal saline as well as dopamine. None of these three patients was given subsequent doses of anguidine. Two additional patients refused to complete their first 5-day course of anguidine because they had experienced severe nausea, vomiting, fever and chills, skin erythema with burning sensation, somnolence, and confusion. In most patients, myelosuppression was mild and occurred between the second and third week of treatment with full hematologic recovery by the third or fourth week.

DISCUSSION

In this population of extensively pretreated patients with advanced metastatic breast cancer, it is perhaps not unexpected that significant antitumor activity was not observed, except for the two patients who showed evidence of response in soft tissue sites. The toxicity observed in our patients was similar to that reported in the initial phase I evaluation (1–6). Nevertheless, this relative lack of antitumor activity and the excessive degree of toxicity in our patients receiving anguidine therapy are somewhat discouraging. The poor patient tolerance associated with anguidine treatment makes this drug less than desirable for the management of patients with advanced malignancies.

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Brief Reports and Preliminary Communications

Phase II Trial of Vindesine in Patients With Malignant Lymphoma[1,2,3]

Robert B. Sklaroff, David Straus, and Charles Young[4,*]

Vindesine (VDS), a semi-synthetic vinblastine (VBL) derivative, possesses an antitumor spectrum against transplanted murine tumors that closely parallels that of vincristine (VCR) (1,2). VDS is less neurotoxic than VCR in vitro, in its effects on axoplasmic transport (3), and in vivo when administered to chickens and rhesus monkeys (4). Pharmacokinetically, VDS resembles VBL rather than VCR (5,6).

In initial clinical studies, VDS produced clinical remissions in patients with acute leukemia, Hodgkin's disease (HD), non-Hodgkin's lymphoma (NHL) (7–10), malignant melanoma (1), testicular cancer, and non-small cell carcinoma of the lung (8). The toxic effects of VDS included myelosuppression, neurotoxicity, alopecia, an acute influenza-like syndrome, and local vascular injury (9). Bone marrow injury was the acute dose-limiting toxic effect; disabling neurotoxicity was uncommon. The present study was designed to examine the usefulness of VDS in heavily pretreated patients with HD and NHL.

MATERIALS AND METHODS

VDS was administered slowly through the side arm of a free-flowing iv infusion in a dosage of 2–4 mg/m^2/week. An adequate trial was defined as one producing therapeutic effect or, in the absence thereof, evidence of dose-limiting toxicity following at least two doses and survival of the patient for at least 1 month. The following response definitions are used: *complete remission (CR)*—a disappearance of all disease for at least 1 month; *partial remission (PR)*—a $> 50\%$ reduction in the summed products of the largest perpendicular diameters of measurable lesions with comparable improvement in abnormal hematologic and biochemical abnormalities for at least 1 month, accompanied by a significant increase in performance status (PS) and a relief of symptoms of disease; *minor regression*—a $> 25\%$ but $< 50\%$ decrease in the sum of the products of the diameters of measurable lesions or tumor regression of greater magnitude which, because of a short duration or associated drug toxicity, was not of clinical benefit.

RESULTS

The characteristics of the patient population entered into this phase II trial are shown in table 1. All patients had received prior vinca chemotherapy; although heavily pretreated, a majority of the patients had a Karnofsky PS that was consistent with therapy on an outpatient basis.

VDS produced one CR, four PRs, and ten minor responses in the 43 patients treated in this study. The CR and the four PRs were obtained in patients with a pretreatment PS of at least 50%. VDS failed to produce clinically useful effects in patients who were incapable of outpatient therapy, although minor evidence of an antitumor effect was noted in two of six low-PS patients with HD and in two of seven low-PS patients with NHL. PRs lasting for 6, 9+, 11+, and 12+ months were obtained in four of 20 high-PS patients with HD. The one patient with NHL who has obtained a CR is a 23-year-old woman

TABLE 1.—Patient characteristics

Parameter	HD	NHL
No. of patients entered/No. adequately treated	31/26	22/17
Median age in yrs (range)	36 (19–69)	53 (22–74)
Sex (male/female)	13/13	10/7
Initial Karnofsky PS (range)	60 (20/100)	50 (20/70)
No. of patients with clinical stage III/IV	3/23	1/16
No. of patients with prior radiation therapy	23 (88%)	11 (65%)
Prior chemotherapy		
Median No. of different drugs (range)	8 (3–13)	7 (4–11)
Median No. of drug regimens (range)	3 (1–6)	3 (1–7)
VCR: No. of patients	25 (96%)	17 (100%)
VBL: No. of patients	22 (85%)	2 (12%)

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[2] Supported in part by Public Health Service grants CA-08748 and CA-05826 from the National Cancer Institute, National Institutes of Health, Department of Health, Education, and Welfare.

[3] Vindesine (Eldisine) was supplied by Eli Lilly and Co, Indianapolis, Ind.

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with diffuse poorly differentiated lymphoma, lymphoblastic type (11). She started to receive the drug at the time of marrow relapse following initial polydrug chemotherapy and continues in CR while receiving biweekly VDS maintenance therapy 12 months following entry into the study.

Ten of 26 HD patients have died; their median survival time from initial VDS therapy was 6 months (range, 2–26 months). Sixteen patients are still alive with a median time at risk of 6 months (range, 3–24 months). Twelve of 17 NHL patients have died; their median survival from initiation of VDS therapy was 4 months (range, 1–10 months). Five patients are still alive with a median time at risk of 8 months (range, 2–21 months).

Myelosuppression, principally leukopenia, was the dose-limiting toxic effect. Leukopenia occurred in 65% of the patients and thrombocytopenia developed in 41%. None of the patients suffered infectious or hemorrhagic complications from therapy. Mild neurotoxicity occurred in 15 patients (35%) and severe neurotoxicity occurred in only three (7%).

Acute influenza-like symptoms, manifested most often by fever and myalgia, were noted in 30% of the patients shortly after the injection. This syndrome was not dose-related; its incidence did not correlate with the occurrence of either the hematologic or the more conventional signs of neurologic toxicity. It was mild and lasted for only 1–3 days after each treatment. Mucositis, not reported in the past, was noted in three patients. The toxic manifestations of VDS were compatible with continued use of the drug. Cumulative myelosuppression did not occur; neurotoxicity required discontinuance of therapy after 2 and 3 months in only two patients.

DISCUSSION

VDS produced four PRs lasting for 6, 9+, 11+, and 12+ months among 20 heavily pretreated patients with HD and one CR lasting for 12+ months among ten NHL patients; no patient with a PS of ≤ 40% responded to VDS. The data suggest that heavily vinca-pretreated lymphoma patients with a good PS will occasionally respond to VDS administered as a single agent. Only two HD patients in this trial were entered in initial relapse following polydrug (MOPP/ABVD)[5] chemotherapy; one obtained a PR that is continuing at 9 months and the other obtained a minor response. The only patient with diffuse histiocytic lymphoma who began receiving VDS therapy after the first relapse from polydrug therapy obtained a CR that is continuing at 12 months.

Although it would be desirable to be able to assess the relative effectiveness of VDS, VCR, and VBL in these disorders, it is not ethically possible to determine a response rate to VDS in patients with HD or NHL who have had no prior exposure to vinca alkaloids because of the great utility of vinca-containing polydrug regimens in the malignant lymphomas. Vinca resistance may not develop consistently during a single program of polydrug chemotherapy. It may be possible to detect greater therapeutic efficacy of VDS if it is employed at the time of first relapse. Further study in this area appears indicated.

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[5] MOPP = mechlorethamine, VCR, prednisone, and procarbazine; ABVD = Adriamycin, bleomycin, VBL, and DTIC.

Combination Chemotherapy for Islet Cell Carcinoma and Metastatic Carcinoid Tumors With 5-Fluorouracil and Streptozotocin [1]

Brief Reports and Preliminary Communications

David Chernicoff, R. M. Bukowski,* Carl W. Groppe, Jr, and J. S. Hewlett [2]

SUMMARY

The results of combination chemotherapy with intermittent courses of 5-fluorouracil and streptozotocin in ten patients with metastatic carcinoid tumors and in eight patients with islet cell carcinoma are reported. Objective responses occurred in two of ten and in two of seven evaluable patients respectively. Biochemical responses occurred in an additional two patients with carcinoid. No improvement over single-agent chemotherapy and no effects on survival were noted.

[Cancer Treat Rep 63:795–796, 1979]

Streptozotocin (STZ) is an antibiotic with a nitrosourea moiety, a methyl group on one end and a glucosamine on the other (1). In bacterial systems it inhibits DNA synthesis, and in animal systems it was found to be destructive to β islet cells (2). STZ has been used in a wide variety of neoplasms, and responses in both malignant carcinoid tumors (3) and functioning and nonfunctioning islet cell tumors of the pancreas (4) have been reported. Experience with other chemotherapeutic agents in these two malignancies has been limited. However, objective responses to 5-fluorouracil (5-FU) in patients with carcinoid tumors (5) and islet cell carcinomas (5) have been reported. With this background data, a phase II trial of these two drugs in combination for the treatment of islet cell carcinoma and metastatic carcinoid tumors was performed.

MATERIALS AND METHODS

Eight patients with unresectable islet cell tumor and ten patients with metastatic or unresectable malignant carcinoid tumor were treated; 16 of these patients had received no prior chemotherapy. In the patients with carcinoid tumor, the location of the primary was in the large or small bowel in nine patients and in the pancreas in one. All patients with islet cell carcinomas presented with pancreatic tumors and no clinical evidence of functioning was noted. Two of ten patients with carcinoid tumor had elevated urinary 5-hydroxyindoleacetic acid and the carcinoid syndrome. Pathologic material was interpreted or reviewed by the Department of Pathology at the Cleveland Clinic Foundation.

Patients were eligible if they had adequate bone marrow function, defined as a wbc count $> 4000/\text{mm}^3$ and a platelet count $> 100{,}000/\text{mm}^3$, and adequate renal function, defined as a BUN of ≤ 20 mg/100 ml and a serum creatinine of 1.5 mg/100 ml.

The chemotherapy used consisted of a 5-day course of STZ and 5-FU given by rapid iv push at a dose of 500 mg/m^2/day, repeated at intervals of 28 days. Patients were considered poor-risk if they were > 70 years of age, had a bilirubin of > 1.5 mg/100 ml, or had received extensive prior radiation to $> 30\%$ of bone marrow-bearing areas and 300 mg/m^2/day of STZ and 5-FU (four patients). A cbc and platelet count were obtained every 14 days, and liver and kidney functions were determined every 28 days. *Complete response* was defined as the absence of clinically detectable tumor. *Partial response* was defined as a decrease by 50% in the product of the two diameters of any measurable lesion, or a 30% reduction in the sum of the liver measurements below the right and left costal margins and xiphoid processes, with no increase in the size of the lesions and no decrease in weight or performance status. *Stable disease* was defined as insufficient regression to be a partial response with no new lesions. *Increasing disease* was defined as a $> 25\%$ increase in the product of the diameters of any measurable lesion or the appearance of new lesions. In two patients with elevated urinary 5-hydroxyindoleacetic acid, quantitation was performed at intervals of 1 month, and a response was defined as a $> 50\%$ decrease compared to pretreatment values. Seventeen patients were evaluable for response and toxicity. One patient with islet cell carcinoma was unevaluable because of failure to complete one course of therapy.

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RESULTS

Partial responses occurred in two of seven patients with islet cell carcinoma and in two of ten patients with carcinoid tumor. The sites of disease in the four responding patients were abdominal. The durations of response were short (30 days) for both responders with islet cell carcinoma and were 130 and 202 days for the two responders with malignant carcinoid. The median survivals for responders with islet cell carcinoma and metastatic carcinoid tumor were 97 days and 312+ days respectively. For nonresponders, these figures were 183+ days and 273+ days respectively. The median survivals for both tumors combined were 210+ days for responders and 152+ days for nonresponders.

The combination of 5-FU and STZ produced moderate toxicity, with all patients experiencing nausea and vomiting. Thrombocytopenia (a platelet count of $< 100,000/\text{mm}^3$) occurred in 7% of the patients and leukopenia (a wbc count of $< 3000/\text{mm}^3$) occurred in 20%. One drug-related death was recorded with life-threatening myelosuppression and renal toxicity. This occurred in the second course of therapy in a patient with islet cell carcinoma. Renal toxicity, as measured by an increase in BUN of > 30 mg/100 ml or an increase in creatinine of > 2 mg/100 ml, occurred in 20% of the patients. No hyperglycemia was noted.

DISCUSSION

In this study, the response rates were 29% for patients with islet cell carcinoma and 20% for patients with carcinoid tumor. If biochemical responses are considered, the overall response rate was 40% in patients with carcinoid.

The experience with pancreatic islet cell carcinoma reported in the literature reveals that with STZ (4) 11 of 30 patients with functioning tumors and three of eight patients with nonfunctioning tumors had objective responses. Biochemical responses in patients with functioning islet cell carcinomas occurred in 25 of 39 patients. Experience with 5-FU therapy for islet cell carcinoma is limited and Moertel (5) reports a response rate of 44% among 16 patients.

Experience with the combination of 5-FU and STZ in metastatic carcinoid tumor has recently been reported by Moertel (6). One hundred and eighteen patients with carcinoid tumor were treated, with a 32% response rate for 5-FU and STZ and a 29% response rate for STZ and cyclophosphamide. If response is determined using both objective measurements and biochemical criteria as in this study, our experience in the ten reported patients is similar.

Results of this study do not appear to differ significantly from the single-agent experience in the treatment of islet cell carcinoma and carcinoid; however, the study was small and uncontrolled. Objective regressions were infrequent, but control of biochemical phenomena did occur. Studies of new agents and the addition of an agent such as cyclophosphamide to the present combination should be considered in future studies of these neoplasms.

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Anaphylactic Reaction to High-Dose Methotrexate[1]

Ilana Gluck-Kuyt[2] and Lowell E. Irwin[3,*]

Brief Reports and Preliminary Communications

The increasing clinical use of high-dose iv methotrexate (MTX) has been associated with several adverse effects. The most common toxic effects of MTX include myelosuppression, ulcerative stomatitis, hemorrhagic enteritis, diarrhea, nephrotoxicity, hepatotoxicity, dermatitis, and alopecia. There have also been case reports of MTX-induced pneumonitis characterized by fever, cough, dyspnea, cyanosis, and bilateral pulmonary infiltrates. Recently, there have been several cases of anaphylactic reactions to high-dose MTX in patients who had previously received bacillus Calmette-Guérin (BCG) (1). The authors of these reports suggested a relationship between BCG and the occurrence of the anaphylactic reaction. This case report describes an anaphylactic reaction to iv high-dose MTX in a patient who had had no prior BCG therapy. A trial of desensitization to MTX was successful for one subsequent course of treatment, but the next course again elicited an anaphylactic reaction.

CASE REPORT

On January 9, 1978, a 12-year-old black male with osteosarcoma was admitted to the Hospital of the Good Samaritan for his 12th course of adjuvant high-dose MTX with citrovorum factor rescue. Ten minutes after initiation of a 6-hour, 6-g infusion, the patient developed hives. MTX was discontinued and iv Benadryl and hydrocortisone were given; the symptoms promptly subsided. The following morning, after premedication with Benadryl and hydrocortisone, MTX was administered without any further adverse reactions. Four weeks later, immediately following initiation of the next course of MTX, the patient developed a generalized sensation of burning, without rash, and vomited. After iv Benadryl and hydrocortisone, MTX was re-started and completed without any further problems. Approximately 6 hours after MTX was completed, the patient complained of facial itching, and the nurse noted peri-orbital edema without rash. The signs and symptoms subsided 4 hours later, after treatment with iv hydrocortisone. One month later, the next course of MTX was begun 1 hour following premedication with iv hydrocortisone. Within 5 minutes, the patient complained of generalized pruritis and the hydrocortisone was repeated. The patient developed diaphoresis, vomiting, and diarrhea and his blood pressure dropped to 42/0 and the apical rate increased to 140. Thirty-five minutes later, vital signs were normal. Two days later, a trial of MTX without a preservative was initiated to determine whether or not the patient was sensitive to the preservative. Again, after only 25 mg of MTX had been given, the patient developed an anaphylactoid reaction with tachycardia to a rate of 160 and diaphoresis. The symptoms subsided shortly after an iv dose of hydrocortisone.

It was now evident that the patient had developed a hypersensitivity to MTX, independent of the preservative. Until this time we had been using an MTX preparation from Lederle Laboratories (Pearl River, NY). We elected to try an MTX preparation from Ben Venue Laboratories (Bedford, Ohio) since the two manufacturers use different methods of production and the drugs produced by each contain different degradation products of the parent MTX compound. Infusion of only 12.5 mg brought about immediate complaints of a burning sensation, for which iv hydrocortisone was administered. Later, an identically colored solution of multiple vitamins (1 ml) was administered iv without any reaction to evaluate the role of any psychosomatic overlay in these reactions. Desensitization to MTX was attempted the next day with the schedule shown in table 1.

Forty-five hours later the infusion of MTX was begun. Within 15 minutes the patient developed generalized pruritis. Thirty minutes after an iv dose of hydrocortisone, the infusion was re-initiated and was completed without any further complications. That evening the patient was given Benadryl for complaints of itching. In May of 1978 the patient was re-admitted for the next course of MTX. Despite premedication with Benadryl, the patient again developed allergic symptoms after 200 mg of MTX had been given. The symptoms subsided 15 minutes after discontinuation of the infusion and treatment with hydrocortisone. Another desensitization trial was initiated on that same day and is also shown in table 1.

Shortly after administration of an MTX test dose, the patient began to complain of itching and developed an-

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TABLE 1.—Desensitization schedule and patient reactions

Time	MTX dose (mg)	Reactions
		1st desensitization trial
11:00 AM	0.1	None
11:10 AM	0.2	None
11:20 AM	0.4	None
11:30 AM	0.8	None
11:40 AM	2.0	None
11:50 AM	4	Itching of scalp
12:00 PM	8	Increased itching involving hands, scalp, and chest
12:10 PM	16	Itching with no redness or rash
12:20 PM	Benadryl	
1:00 PM	4	None
1:10 PM	8	Patient fell asleep
1:20 PM	16	Still asleep
1:35 PM	25	No reaction
		2nd desensitization trial
12:00 PM	0.5	None
12:10 PM	1.0	None
12:20 PM	2.0	None
12:30 PM	4.0	None
12:40 PM	8.0	None
12:50 PM	16	None
1:00 PM	25	None

aphylactoid symptoms with tachycardia and hypotension. The administration of hydrocortisone resulted in resolution of the reaction.

DISCUSSION

This case report illustrates an anaphylactoid reaction to MTX. The patient had tolerated his previous courses of high-dose MTX without any complications. His first adverse reaction developed upon initiation of his 12th course of therapy. Each time the patient was admitted, iv hydration was initiated 24 hours prior to the MTX infusion and was maintained throughout the course. An alkaline urine pH was also maintained throughout each course. Renal and liver functions and cbc remained within normal limits throughout the entire course of chemotherapy.

MTX without the preservative, benzyl alcohol, also elicited an anaphylactic reaction with tachycardia and hypotension. Since Lederle and Ben Venue Laboratories produce MTX by different methods and the final products contain different degradation products, we elected to use both preparations of MTX. *N*(10)-methylfolic acid; 2,4-diamino-*N*-10-methylpteroic acid; 2,4-diamino-*N*-10-methylpteroic amide; and 4-amino-4-deoxy-*N*(10)-methylpteroyl-*N*-methylglutamine are some of Lederle's degradation contaminants that have been identified. Another case report sites a patient who experienced anaphylactoid reactions to Lederle's preparation of MTX but none to Ben Venue's (1). Our patient developed anaphylactoid reactions to both manufacturers' products.

Successful desensitization with increasing doses of MTX was carried out for one course of chemotherapy, but the patient became refractory upon the subsequent course. A second attempt at desensitization with MTX was unsuccessful.

Two other cases of anaphylactoid-type reactions to high-dose MTX have been reported, but both patients were receiving concomitant intracutaneous BCG. It was suggested by the investigators (1) that "the possibility exists that BCG, acting as an immunologic adjuvant, potentiated the development of an immune reaction to methotrexate or contaminants of the preparation." Our patient experienced several episodes of anaphylactoid reactions to high-dose MTX without any prior immunization with BCG. Desensitization was possible for one course of MTX but not for a subsequent course.

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ADDENDUM

Since the submission of this manuscript there has been an additional case of an anaphylactic reaction in a patient receiving her eighth course of high-dose MTX without any prior BCG therapy (report of J. Bonorris, MD, Hospital of the Good Samaritan, Los Angeles, Calif).

Design and Evaluation of Combination Chemotherapy Trials in Experimental Animal Tumor Systems[1,2]

Brief Reports and Preliminary Communications

T. H. Corbett,[3] D. P. Griswold, Jr,[3,*] Mary K. Wolpert,[4] John M. Venditti,[4] and Frank M. Schabel, Jr[3]

The treatment of tumors in man with combinations of chemotherapeutic agents has, with few exceptions (eg, Burkitt's lymphoma and choriocarcinoma), become common practice. Many of the combinations have been selected on an arbitrary basis, although usually from among active single agents. Because of the overwhelming number of possibilities involved in the design of combination chemotherapy trials, chemotherapy results from animal tumor systems have been increasingly used to provide a stronger basis on which to select agents, dose regimens, sequence, etc. Two marked advantages of using animal tumor models for this purpose are (*a*) the relatively short time required for animal tumor studies and (*b*) the ability to properly control all aspects of each trial, whereas in therapy trials in man, absolute control of variables is limited by ethical and moral constraints.

Some reports of combination chemotherapy results at recent meetings may have implied unrealistically that a combination of agents was superior in antitumor activity to any of the single agents included in the combination. Such conclusions are allowed when adequate controls are not included in experimental combination chemotherapy trials. The purpose of this report is not to question the potential superiority of any particular combination of agents but to suggest that presentation of experimental data should include sufficient information, including results from single-agent control groups, so that the viewer (or reader) can have the opportunity to evaluate the data objectively.

MATERIALS AND METHODS

Combination chemotherapy experiments were undertaken using mouse colon tumors 26 and 38. These tumor systems and methods employed in chemotherapy trials have been previously described (1). Briefly, a 20–40-mg tumor fragment was implanted sc into each of a number of BDF_1 (C57BL/6 × DBA/2) (colon tumor 38) or CDF_1 (BALB/c × DBA/2) (colon tumor 26) mice. When the tumors reached approximately 200–400 mg, mice with tumors outside that range were discarded and the remainder were randomly distributed into treatment and control groups. Treatment was begun immediately as indicated in tables 1 and 2. Tumors were measured by caliper twice weekly and mice were observed daily for deaths. Mice without tumors were included for drug toxicity controls.

RESULTS AND DISCUSSION

In the first example (table 1) it can be seen that 5-fluorouracil (5-FU) and CCNU in combination (groups 10 and 11) were clearly superior in antitumor effectiveness to either single agent (groups 2 and 6) *at equitoxic doses*. This observation is validated by the presence of single-agent groups (1 and 5) that had unacceptable lethal drug toxicity, while the next lower doses, with acceptable toxicity, were not as effective against the tumor as the combination.

In the second example (table 2), had the data from only groups 4, 8, and 11 been presented, the erroneous conclusion might have been drawn that the combination of *cis*-dichlorodiammineplatinum(II) (*cis*-DDP) plus methyl-CCNU (meCCNU) was superior to either single agent. Comparison at equitoxic doses (group 11 versus group 6), however, shows that the combination was not clearly more effective than meCCNU alone.

Drug toxicity determination is an important and necessary part of chemotherapy trials. The LD10 has traditionally been accepted by experimentalists as the maximum-tolerated dose and provides a basis for comparison of the effects of one treatment with another. A therapeutically synergistic combination has been defined as one that produces a therapeutic response which is superior to the maximum response obtainable by either agent alone (2,3). The phrase "at equitoxic doses" should be added. Furthermore, experimental therapy trials

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TABLE 1.—Chemotherapy for sc implanted colon tumor 38 using 5-FU and CCNU as single agents and in combination*

Group	Agent(s)	Dosage (mg/kg/day)	Median % ILS†	No. of mice with tumor regression/No. treated		Tumor growth delay (days, T–C)‡	Tumored drug deaths§	Nontumored control drug deaths§
				Partial	Complete			
1	5-FU	141	–22	0/2	0/2	18.7	8/10	10/10
2	5-FU	95	19	0/9	0/9	13.5	1/10¶	0/10
3	5-FU	76	38	0/10	0/10	14.4	0/10	0/10
4	5-FU	62	43	0/10	0/10	7.3	0/10	0/10
5	CCNU	33	36	0/8	0/8	18.1	2/10	1/10
6	CCNU	22	18	0/10	0/10	10.8	0/10¶	0/10
7	CCNU	15.4	36	0/10	0/10	6.9	0/10	0/10
8	CCNU	12.1	8	0/10	0/10	5	0/10	0/10
9	5-FU CCNU	95 22	–26				10/10	10/10
10	5-FU CCNU	80 15.4	94	3/9	4/9	30.5	1/10¶	0/10
11	5-FU CCNU	76 15.4	109	4/10	1/10	29.1	0/10	0/10

*Treatment in all cases was q7days × 3, beginning 14 days after tumor implantation. 5-FU was given iv and CCNU was given orally. There were no tumor-free survivors in any groups. A partial response was recorded when a tumor regressed by > 50% and a complete response was recorded when a tumor became unpalpable.

†ILS = increase in lifespan.

‡T–C = difference (days) for treated and untreated tumors to reach a given size (1250 mg). C = 23 days; control median day of death = 35.5 (no tumor-free survivors or regressions were observed among the 20 untreated tumor control mice).

§No. of mice dying/No. treated.

¶Maximum-tolerated dose.

TABLE 2.—Chemotherapy for sc implanted colon tumor 26 using *cis*-DDP and meCCNU as single agents and in combination*

Group	Agent(s)	Dosage (mg/kg/day)	Median % ILS†	No. of mice with tumor regression/No. treated		Median duration of regression (days)	No. of tumor-free survivors/ No. treated	Tumor growth delay (days, T–C)‡	Tumored drug deaths§	Nontumored control drug deaths§
				Partial	Complete					
1	*cis*-DDP	11	10	1/1	—	13.6	0/10	31.5	9/10	10/10
2	*cis*-DDP	7.1	100	3/9	2/9	11.1	1/9	24.3	1/10¶	2/10
3	*cis*-DDP	4.6	82	0/10	1/10	—	1/10	9.6	0/10	2/10
4	*cis*-DDP	2.9	100	0/10	1/10	—	1/10	2.8	0/10	0/10
5	MeCCNU	25	82	0/3	3/3	—	3/3	—	7/10	4/10
6	MeCCNU	16.5	173	2/8	6/8	25.9	2/8	32.8	1/9¶	0/10
7	MeCCNU	11	78	3/10	3/10	5	3/10	13.6	0/10	0/10
8	MeCCNU	7	86	3/10	1/10	12.6	1/10	13.3	0/10	1/10
9	*cis*-DDP MeCCNU	4.6 7	186	3/8	5/8	27.3	1/8	47.7	2/10	3/10
10	*cis*-DDP MeCCNU	2.9 11	134	1/6	5/6	31.4	1/6	29.1	4/10	2/10
11	*cis*-DDP MeCCNU	2.9 7	110	6/9	3/9	18.4	0/9	35.6	1/10¶	0/10

*Treatment in all cases was q7days × 3, beginning on Day 10. *cis*-DDP was given iv and meCCNU was given orally. *See* table 1 for further details.

†ILS = increase in lifespan.

‡T–C = difference (days) for treated and untreated tumors to reach a given size (1250 mg). C = 20 days; control median day of death = 23 (no tumor-free survivors or regressions were observed among the 19 untreated tumor control mice).

§No. of mice dying/No. treated.

¶Maximum-tolerated dose.

should be so designed that they can be readily interpreted from this standpoint.

Claiming therapeutic synergism for a two-drug combination in which the dose of either drug used in the combination is less than the optimal therapeutic dose of either single drug at ≤ the LD10 level is neither logical nor indicated, particularly if the optimal therapeutic dose of either drug when used alone is equal to or greater in therapeutic activity than that of the drug combination alleged to be therapeutically synergistic. In addition, LD10 doses of anticancer drugs as determined in non-tumor-bearing animals may exceed the LD10 doses of the same drugs in animals bearing advanced tumors. Thus, great care must be used in interpreting equitoxic doses of single and combination drugs in comparative therapy trials.

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Occurrence of Testicular Metastasis in a Child With Bilateral Retinoblastoma[1]

Brief Reports and Preliminary Communications

Jim C. Kimball and Ayten Cangir[2,*]

Retinoblastoma is the most common intraocular tumor occurring in children. It has attracted the attention of both geneticists and epidemiologists because of the importance of genetic factors in its etiology and the occurrence of second malignancies in those children with a genetic predisposition (1–5). Metastatic disease may develop in both genetic and nongenetic forms by direct extension, lymphatic spread, or hematologic spread. The most common sites of metastasis from retinoblastoma, in decreasing order of frequency, are orbit and periglobal tissues, cranium, central nervous system and intracranial deposits, bone marrow, distant bones, and viscera. Lymph node involvement may accompany any of the above or may occur separately. The patient described in this report represents the first known example of retinoblastoma metastatic to the testis.

CASE REPORT

A 35-month-old black male with a diagnosis of bilateral retinoblastoma had enucleation of the left eye in July 1975. Chemotherapy consisting of three doses of vincristine (2 mg/m^2) and two doses of cyclophosphamide (40 mg/kg) was given prior to the administration of radiation therapy. From September to October 1975, the patient received a 3550-rad midline dose through parallel-opposed fields using 18-mev photons, with a 375-rad boost to the right eye. Radiation fields extended posteriorly to include the optic chiasma due to suspected involvement of the optic nerve (only a small portion was microscopically examined). Prophylactic intrathecal chemotherapy was concomitantly administered (initially, hydrocortisone and cytosine arabinoside were alternated with hydrocortisone and methotrexate during "induction"; subsequently, all three drugs were given intrathecally every 8 weeks). Intrathecal chemotherapy was continued upon completion of the radiation even though the central nervous system remained free of metastatic disease. The right retinal lesion regressed and remained stable, as evidenced by serial examinations under anesthesia, until hypopyon developed in September 1976. Anterior chamber paracentesis revealed the presence of retinoblastoma cells. Evaluation for evidence of additional metastatic disease was negative. Enucleation of the right eye was performed.

One month postoperatively, a hard, nonpainful mass was felt in the right mid-mandibular region. A biopsy specimen showed metastatic retinoblastoma infiltrating the bone, muscle, lymph node, and fibroadipose tissues; electron microscopic confirmation was obtained. Chest radiograms revealed a pleural mass with associated right posterior fourth-rib changes. Further metastatic evaluation revealed two lytic lesions of the left femur. Systemic chemotherapy with monthly Adriamycin (45 mg/m^2 iv on Day 1), vincristine (1.5 mg/m^2 iv on Day 1), cyclophosphamide (7 mg/kg iv on Days 1–5), and 5-fluorouracil (8 mg/kg iv on Days 1–5) was initiated, but doses had to be reduced subsequently by 25% due to severe myelosuppression. Prophylactic intrathecal drugs were continued on a bimonthly schedule. When a cumulative dose of 430 mg/m^2 of Adriamycin was reached, actinomycin D (15 μg/kg iv) was substituted.

By December 1976, the chest mass had disappeared and there was evidence of healing of the mandibular lesion. In January 1977, a followup bone survey was negative for evidence of metastatic disease. In April 1978, there was radiographic suspicion of recurrence of disease within the right mandible as evidenced by cortical thinning and elevation of deciduous teeth. This was diagnosed later as a pyogenic abscess. Additional investigations performed at this time included bone scan, bone survey, bone marrow aspirate, examination of cerebral spinal fluid cytology, and chest radiogram; all of these were negative for evidence of disease. On physical examination however, a rock-hard, nontender left testicle measuring 1 × 2 × 2 cm was palpated. A left orchiectomy demonstrated metastatic retinoblastoma within the testis; electron microscopic confirmation was obtained.

Systemic chemotherapy was changed to high-dose methotrexate (up to 200 mg/kg, 6-hour infusion) with citrovorum factor rescue and vincristine (1 mg/m^2) on a triweekly schedule. In August 1978, the patient died from a choking incident while at home during a convalescent period from an upper respiratory infection; permission for an autopsy was denied.

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DISCUSSION

Extraocular metastatic disease heralds a very poor prognosis (6). Among the 505 children with retinoblastoma followed for at least 10 years after their diagnosis by Kitchin and Ellsworth (7), 15% of those with unilateral disease died from metastatic spread, while 11% of those with bilateral disease died from metastasis. In a series of 168 patients analyzed by Lennox et al (4), only one of 40 children who developed metastasis survived. It is of interest that much attention has been directed to the testes as an immunologically privileged site, a "sanctuary" for recurrence of disease, especially in children with acute leukemias (8). Furthermore, there are suggestions that the incidence of leukemic testicular involvement increases with the use of certain chemotherapeutic agents, particularly, cyclophosphamide and cytosine arabinoside (9,10). If the testes are a pharmacologic sanctuary site for tumor cells and if metastatic spread can be hematogenous, it seems unusual that testicular metastases have not been noted before in children with retinoblastoma. As effective therapeutic regimens are developed to control and eradicate metastatic disease, close attention must be directed to all readily observable organ systems.

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LETTERS

New Polychemotherapy Regimen for Head and Neck Tumors: Pilot Study

Because current local treatment methods for the management of head and neck tumors have not given definitive results, we have studied a new systemic polychemotherapeutic regimen (conducted within the framework of a wider study project) aimed at developing a new treatment protocol (1,2). A homogeneous group of head and neck cancer patients were treated as follows: cyclophosphamide, 3 mg/kg iv twice a day on Days 1 and 2; hydroxyurea, 50 mg/kg/day orally on Days 3–6; treatment suspended on Days 7 and 8; vinblastine, 0.1 mg/kg iv on Day 9; and vincristine, 0.01 mg/kg iv on Day 9. Two treatment courses were administered at 15-day intervals. The clinical investigations described below were carried out after completion of the second course.

Fourteen patients were treated (ten males and four females), all of whom had histologically proven squamous cell carcinoma of the head and neck in progression. The disease was localized as follows: tonsils, two patients; pharynx, four; tongue, six; floor of the mouth, infiltrating the submandibular gland, one; and soft palate, one. The median age was 61 years (range, 39–76 years). The Karnofsky performance status was $\geq 30\%$ and all patients had a life expectancy of at least 2 months and had not received cancer treatment for at least 1 month. Blood and biochemical investigations were carried out on all patients to assess bone marrow, liver, and kidney function. Evaluation criteria were as follows: *partial response*—$> 50\%$ reduction of the surface area of all measurable masses; *no change*—no changes in lesions or reduction of $< 50\%$ in some lesions with no change in others; and *progression*—increase of even one lesion by $> 25\%$ or appearance of new metastases.

RESULTS

Leukopenia was observed in nine of 14 patients, with wbc values $< 1500/\text{mm}^3$ in only one patient (table 1). Nausea was the only clinical side effect recorded and affected one patient for 7 days (7%). No statistically significant alterations were recorded in any of the other clinical investigations carried out, such as rbc, wbc, and platelet counts, hemoglobin, urea, glucose, creatinine, uric acid, bilirubin, SGOT, SGPT, alkaline phosphatase, prothrombin time, and electrophoresis of serum proteins.

TABLE 1.—Leukopenia (observed in 9 of 14 patients)

	1st course	2nd course
Median duration in days (range)	4 (2–20)	7 (2–21)
No. of patients with wbc counts (cells/mm^3) of—		
4000–3000	4	2
2900–1500	4	7
< 1500	1	—

TABLE 2.—Objective remissions

Response	No. of responses/ No. of patients treated
Partial remission	6/14 (43%)
No change	6/14 (43%)
Progression	2/14 (14%)

Table 2 shows the objective remission data. There were no complete remissions. A partial remission was observed in six of 14 patients (43%) and lasted an average of 3.5 months (range, 2–8 months). Five of seven patients (83%) experienced significant pain relief or disappearance of pain altogether. Median duration of relief from pain was 30 days (range, 30–45 days). The duration of objective response was short, which is characteristic of squamous cell carcinoma of the head and neck; nevertheless, these results should encourage us to further our studies into the systemic treatment of this type of tumor with this regimen.

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Reversible Renal Dysfunction Due to 5-Azacytidine

The pyrimidine analog 5-azacytidine (5-AZC) has been shown to be effective in the treatment of patients with acute myelogenous leukemia (1,2). Although preclinical studies of its toxicity in dogs (3) and monkeys (4) predicted an effect on the kidneys, renal toxicity has not previously been reported in patients treated with 5-AZC. This report is prompted by the observation of azotemia in four consecutive courses of 5-AZC given to three patients with acute leukemia.

Each patient was given 5-AZC as a continuous iv infusion for 5 days at a dose of 150 mg/m^2/day. An appropriate quantity of the drug was dissolved in 100 ml of Ringer's lactate and was infused over a 3-hour period. Fresh solution was prepared every 3 hours. One patient was given a second 5-day course of the drug at a dose of 200 mg/m^2/day. None of the patients had a partial or complete remission with this treatment.

Patient 1 was a 65-year-old man with acute myelogenous leukemia in whom a complete remission was initially induced with cytosine arabinoside and Adriamycin. Despite maintenance chemotherapy every 8 weeks, he relapsed after 8 months. He was then treated with 5-AZC because of severe neutropenia associated with a hypercellular bone marrow in which 27% of the nucleated cells were myeloblasts. He received two courses of 5-AZC. During the initial course (150 mg/m^2/day) the serum creatinine rose from 1.5 to 1.9 mg/dl. During the following 12 days, the serum creatinine fell to 1.2 mg/dl. Because of the persistence of a leukemic marrow, a second course of 5-AZC (200 mg/m^2/day) was given, during which the serum creatinine rose to 3.1 mg/dl. Again, 12 days after the 5-AZC infusion, the serum creatinine fell gradually to 1.2 mg/dl. The following drugs were given during the first course of 5-AZC: allopurinol each day and gentamicin and oxacillin for 3 days only (Days 3–5). Only allopurinol was given during the second course of 5-AZC. The serum uric acid concentration did not rise during either course.

Patient 2 was a 63-year-old woman with acute myelomonocytic leukemia who relapsed 30 months after a complete remission was induced and maintained with cytosine arabinoside and 6-thioguanine. After recovery from an ineffective 3-day course of Adriamycin, she was treated with 5-AZC for 5 days. She died of sepsis on the day after completion of this therapy. During the administration of 5-AZC there was a daily progressive rise in serum creatinine from an initial concentration of 1.2 mg/dl to 2.3 mg/dl on the day of death. During this period of time the patient was treated with allopurinol and there was no elevation in the serum uric acid. She was also treated concomitantly with gentamicin and oxacillin.

Patient 3 was a 68-year-old man with acute monocytic leukemia. Three induction courses of cytosine arabinoside and Adriamycin induced a partial remission from which he relapsed despite maintenance treatment with cytosine arabinoside and 6-thioguanine. Because of a rapidly rising wbc count to levels $> 100{,}000/\text{mm}^3$, he was treated with 5-AZC. During this course of treatment, his serum creatinine concentration rose progressively from 1.2 mg/dl to 2.7 mg/dl on the fourth day when he died of sepsis. There was no elevation in serum uric acid concentration during the treatment period. Urinalysis showed 70 mg of protein/dl on the day prior to the start of the 5-AZC infusion and 139 mg/dl on the second day of treatment. Allopurinol, gentamicin, and oxacillin were given concomitantly during the course of 5-AZC therapy.

The observations in patient 1 suggest that the serum creatinine elevation was, in fact, primarily due to 5-AZC and that the impairment in renal function is reversible. The data concerning the other two patients are also consistent with renal impairment due to 5-AZC. However, infection and concomitant use of other drugs with a potential for renal toxicity offer alternative explanations for the renal dysfunction. At postmortem examination there was interstitial leukemic infiltration in the kidneys of patients 2 and 3, another possible cause of renal dysfunction. No autopsy was done on patient 1.

5-AZC therapy should be considered one of the risk factors for renal toxicity in patients with acute leukemia.

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Proceedings of the NCI-EORTC Symposium on New Drugs in Cancer Therapy

Institut Jules Bordet

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Introduction: Anthracycline Derivatives in New Drug Development Programs

Marcel Rozencweig,[1,*] Christian De Sloover,[2] Daniel D. Von Hoff,[1] Henri J. Tagnon,[3] and Franco M. Muggia[1,4]

The National Cancer Institute-European Organization for Research on Treatment of Cancer Symposium on New Drugs in Cancer Therapy provided an opportunity to integrate various programs in drug development with contributions from Europe, Japan, and the US. Part of the meeting was devoted to the problem of analogs, with special emphasis on anthracycline derivatives. The proceedings in this issue focus on preclinical and clinical aspects of a field that has shown extreme productivity.

Investigations of compounds structurally related to active anticancer agents represent a major aspect of new drug development programs. This area of research has produced numerous drugs for experimental testing in various classes of chemotherapeutic agents. Few of these drugs have been selected for clinical trials and, often, their initial promise could not be confirmed or conflicting results precluded firm conclusions.

Various relationships may be defined between analogs (fig 1). In fact, it may be difficult to identify these relationships with accuracy because of practical limitations in the clinical investigation of anticancer drugs. Differences in pharmacology and/or cytotoxic selectivity must be of pronounced magnitude to make clinical benefits detectable. Hence, a carefully planned order of priorities and strong rationales are prime requirements before embarking on clinical trials with new analogs.

Several rationales are employed to introduce related compounds into clinical trials. In general, the preclinical avenues for developing analogs become more extensive as knowledge is acquired on clinical capabilities and toxic effects of an anticancer agent. Thus, whereas use of Adriamycin followed use of daunorubicin presumably based on a somewhat better antitumor activity in mice, analogs are now being considered to warrant further testing because of: (*a*) altered pharmacokinetics, (*b*) altered toxicity to heart, integument, or bone marrow, and (*c*) better therapeutic index as defined by maintenance of antitumor activity relative to one or more of the above toxic effects.

The selection of an analog for further testing becomes increasingly complex as more experimental data to make this choice are considered. In fact, the validity of such a choice, or the models on which it is based, short of confirmation in clinical testing, cannot be ascertained. This latter point requires special attention.

Perplexing ways have often characterized the clinical investigation of analogs. It may take years of experience with both the parent drug and the analog before the relevant questions are asked and eventually answered. Efforts to compare these drugs with parent compounds have been rendered difficult by the "softness" of certain clinical data. On the other hand, comparative randomized trials require a vast number of patients and are often unattractive in most clinical settings. In this symposium, this search for solutions is particularly apparent because of the interest in anthracyclines and, it would appear, because of the large number of analogs proposed for our consideration.

A few basic guidelines can be recommended for large-scale clinical studies with anthracycline derivatives when early clinical trials do not demonstrate any advantage in terms of pharmacologic characteristics (eg, feasibility of oral administration) or in terms of acute and subacute toxic effects (eg, lack of alopecia or vesicant properties). Activity in solid tumors that are inherently resistant to Adriamycin such as melanoma or colon cancer is worth seeking and perhaps easiest to establish. Agents some-

Cross Resistance	Relationship		(Example)
Absolute	Intimate	1. Prodrug/drug	(Azotomycin/DON) (Ftorafur/5Fu)
	↓	2. Drug/Metabolite; shared active moiety	(Hexa/PentaMM) (5Fu/5FuDR)
	↓	3. Same targets	(MTX)/dichloro MTX)
Occasionally circumvented	↓	4. Probably similar targets	(VP-16/VM-26) (adria/dauno)
Relative	↓	5. Similar, with some different targets	(DON/azaserine) (aclacino/adria)
No cross-Resistance	↓	6. Chemically related, but different targets	(5Fu/ara-C) (chloro/streptozotocin)
	Remote		

FIGURE 1.—Examples of variable relationships between analogs.

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[4] The help of N. Helms and D. Eeckhoudt in the organization of this symposium is greatly appreciated.

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what less chemically related to Adriamycin or daunorubicin and unlikely to act like a prodrug of the latter could be considered for trial in patients previously treated with these agents provided that the estimated risk of cardiotoxicity is kept within acceptable limits.

Carefully designed comparative trials give the ultimate answer in the determination of relative efficacy. In breast cancer these trials appear most feasible, since Adriamycin as a single agent is an optimal second-line treatment. Along this line, a comparative trial of Adriamycin and rubidazone is in progress in advanced breast cancer at the Mayo Clinic, Rochester, Minn. In addition, sarcomas may be suitable for single-agent comparative trials if some activity is detected with an analog in these diseases. Trials in combination with other drugs are feasible in leukemia or lymphoma and have been initiated only recently for the two initial anthracyclines.

Comparison of toxic manifestations must take into account the dose and method of administration and scheduling. For example, it is always possible to demonstrate lower toxic effects with an analog at doses which do not achieve equivalent myelosuppression. Longer infusion rates may lessen toxic effects of the parent compound as well.

Efforts in the development of anthracycline derivatives are primarily directed to the detection of compounds endowed with significantly lesser cardiotoxicity, not in terms of mg/kg but relative to cumulative therapeutic doses. A clinical comparison of anthracyclines based on the occurrence of congestive heart failure (CHF) can hardly be undertaken. CHF is related to the total cumulative dose administered and is frequently irreversible and lethal. The cumulative probability of developing drug-induced CHF with Adriamycin is relatively rare (7%) at doses of ≤ 550 mg/m^2, and this probability might be greatly modulated by a number of variables including dose schedule of administration and age of the patient (1).

Monitoring for cardiotoxicity is an essential facet of the clinical investigation of new anthracyclines. Endomyocardial biopsy as well as a variety of noninvasive techniques have been advocated in this endeavor (2,3). Sampling of endomyocardial tissue and interpretation of pathologic lesions still need additional refinements to improve the reliability of this approach. Noninvasive techniques have usually not been useful in predicting the appearance of cardiotoxicity in *individual* patients. However, some abnormalities encountered with Adriamycin have been suggested to be dose-related which would allow comparative studies of cardiotoxic potential in *groups* of patients.

Other papers in this issue summarize preclinical data of possible relevance for selecting anthracycline derivatives for clinical use. This background information introduces an overview of the clinical experience accumulated with DNA complexes, quelamycin, rubidazone, AD-32, carminomycin, 4′-epi-adriamycin, diethoxy-1-acetoxy-14-daunorubicin, and aclacinomycin A. These compounds have been investigated to various extents but, to date, a clear advantage over daunorubicin and Adriamycin remains to be demonstrated with respect to both antitumor activity and spectrum of toxicity.

Presently available results emphasize the difficulties in the clinical development of anthracycline derivatives. Future clinical trials must take heed of the above guidelines in demonstrating a place for an anthracycline analog in our therapeutic armamentarium. Analog development must rely on valid preclinical rationales which, in turn, are also dependent upon appropriate feedback from clinical studies. The design of these studies may require even greater sophistication than studies with drugs heralding new classes of chemotherapeutic agents.

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Daunorubicin and Adriamycin in Cancer Treatment: An Analysis of Their Roles and Limitations[1]

Hugh L. Davis* and Thomas E. Davis[2]

Thirteen and 10 years respectively have elapsed since daunorubicin and Adriamycin were introduced into clinical trials. This period has been a time of intense clinical and laboratory investigation in cancer treatment marked by the successful application of combination chemotherapy, the application of the principles of combined modality therapy, and the effective use of ancillary medical support, notably blood component therapy and treatment of opportunistic infections. These two anthracycline antibiotics have had a major impact on cancer chemotherapy, yet they share unique as well as general limitations which pose major obstacles to further improvement in therapy.

During the last 3 years, there have been 305 abstracts published in the combined Proceedings of the American Association for Cancer Research and the American Society of Clinical Oncology which encompass the role and limitations of therapy with these agents. This paper will assess some current achievements and limitations uncovered by these prolific investigations.

As a background to these selected issues the single and combination antitumor activity of Adriamycin is presented in table 1. Adriamycin is probably the most "broad-spectrum" single agent in cancer therapy (1–3). The spectrum of activity of daunorubicin is currently believed to be narrow, confined mainly to acute leukemia (4), although investigations in solid tumors have been meager and largely unevaluable (5). Most combination trials with Adriamycin have been disease-oriented, although combinations with 5-fluorouracil (5-FU) (6), cyclophosphamide (7), and *cis*-dichlorodiammineplatinum(II) (DDP) (8) have received broad phase II trials with activity discovered in specific neoplasms.

OPTIMUM DOSE, SCHEDULING, AND DURATION OF THERAPY

These general issues of anthracycline therapy are closely linked with the major cumulative toxicity, total dose-dependent cardiomyopathy (9,10). Retrospective studies have shown that both anthracyclines demonstrate a rising probability of potentially fatal cardiotoxicity at a cumulative dose of > 600 mg/m^2 for daunorubicin and > 550 mg/m^2 for Adriamycin (10). It has been emphasized by Von Hoff et al (9,11) that there is no precise cutoff point for total dose limitation since there is a continuum of risk and increasing dose.

Noninvasive methods of predicting the development of congestive heart failure (CHF) including electrocardiographic voltage reduction, systolic time intervals, and echocardiography have not been universally successful in monitoring risk since cardiac failure may abruptly occur after repeatedly normal studies (10). The recommendations have been to restrict the total lifetime dose to arbitrary "safe" levels, leaving the clinician with the dilemma of risk versus benefits of continued therapy.

A few studies of dose response and scheduling have attempted to determine ways of safely prolonging the total period of Adriamycin exposure. A broad phase II study of dose response in q3week intermittent schedules was reported by O'Bryan et al (12) for the Southwest Oncology Group (SWOG). Good-risk versus poor-risk patients (based on clinical evaluation by the investigator) were randomly allocated to receive doses varying from 75 to 45 mg/m^2 for good-risk patients and 50 or 25 mg/m^2 for poor-risk patients. The results are summarized in table 2. Note that sarcomas probably have the steepest dose-response curve, a finding also suggested by a Cancer and Leukemia Group B (CALGB) osteosarcoma adjuvant study where treatment failure increased if dose reductions occurred (13) and by an Eastern Cooperative Oncology Group (ECOG) study where maximum responses occurred at doses of 70 mg/m^2 q3weeks (14). The lack of dose response in lymphoma is noteworthy. The shallow curve in breast cancer has been exploited by Creech et al (15), who have compared low-dose to high-dose Adriamycin and noted a similar remission rate. A disquieting conclusion of O'Bryan's study was that CHF could not be eliminated by total dose limitation to 550 mg/m^2; five irreversible cases occurred at doses below that level. This agrees with Von Hoff's statement that risk is a continuum with no absolute safe dose levels (9,11).

Studies by the Central Oncology Group (COG) (16) and by Weiss and Manthei (17) used a brief loading course followed by weekly maintenance injections. The COG

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TABLE 1.—Antitumor activity of Adriamycin by tumor site

Major clinical utility	Definite activity, extent to be defined	Minor or no activity	Unevaluable
Acute leukemia Lymphocytic Nonlymphocytic	Multiple myeloma	Large bowel	Esophageal
Childhood tumors Wilms' Neuroblastoma Rhabdomyosarcoma Ewing's sarcoma Osteogenic sarcoma	Lung cancer Small cell Adenocarcinoma Large cell Squamous cell	Pancreas	Brain tumor
Hodgkin's disease	Head and neck squamous cell carcinoma	Melanoma	
Non-Hodgkin's lymphoma	Gastric carcinoma	Renal adenocarcinoma	
Breast carcinoma	Hepatoma		
Soft tissue sarcoma	Gynecologic carcinoma Ovary Uterus Cervix		
	Genitourinary carcinoma Bladder Prostate Testis		
	Endocrine cancer Thyroid Islet cell tumors		
	Carcinoid		
	Mesothelioma		

TABLE 2.—Evaluation of dose response to Adriamycin in solid tumors (SWOG)*

	Dose
Study design	
Good-risk patients	75 vs 60 vs 45 mg/m^2 iv q3wks
Poor-risk patients	50 vs 25 mg/m^2 iv q3wks
Overall response rates (CR + PR)	
Good-risk patients	75 mg/m^2 (66 of 263 patients, 25%); 60 mg/m^2 (26 of 95 patients, 27.4%); 45 mg/m^2 (14.4%)
Poor-risk patients	50 mg/m^2 (21 of 131 patients, 16.0%); 25 mg/m^2 (17 of 138 patients, 12.3%)

*Conclusions:

1. A shallow dose-response curve was identified.
2. No statistically significant differences in rate and duration of response were identified.
3. 75 mg/m^2 was advantageous in terms of rapid induction of response (good-risk patients).
4. Good-risk patients with sarcoma, bladder carcinoma, and prostate carcinoma may respond best to a dose of 75 mg/m^2. Poor-risk patients were generally unresponsive.
5. Breast cancer did not follow a dose-response curve in good-risk patients, but 50 mg/m^2 was superior to 25 mg/m^2 in poor-risk patients.
6. There was no dose-response curve for patients with malignant lymphoma.
7. 90% of patients achieving remission did so by the third dose.
8. 13 of 818 patients (1.6%) developed drug-related CHF at doses ranging from < 200 to 550 mg/m^2.

studied Adriamycin as a single agent; as of July 1976, 75 of 442 patients had received a dose of > 550 mg/m^2, with only six cases of possible cardiomyopathy. Weiss' update adding his personal experience indicated possible cardiomyopathy in eight of 149 patients at a dose of > 550 mg/m^2. Much of these data received a retrospective review by Von Hoff et al (11) who found significantly less cardiotoxicity with the weekly regimen compared to the q3week schedule. The COG study observed response rates in the major sensitive tumors comparable to those of other studies. However, no prospective current data are available to confirm this. The ECOG has begun a protocol testing Adriamycin at a dose of 70 mg/m^2 q3weeks versus low-dose weekly Adriamycin (as studied by the COG) versus Adriamycin and DTIC (as previously reported by the SWOG). Thus, the ECOG study will prospectively attempt to answer both efficacy and safety questions and will potentially require a large case accrual.

Thus, prevention of cardiotoxicity and optimum dose and schedule of Adriamycin remain major clinical issues.

COMPARATIVE EFFICACY OF ANTHRACYCLINES IN COMBINATION IN SENSITIVE TUMORS

A comparison of trials designed to test the efficacy of an anthracycline alone or in combination against other

TABLE 3.—MOPP vs ABVD in previously untreated Hodgkin's disease: Milan studies

Stages	No. of patients	Treatment programs	Results (% CR)	% in CR
IIB, IIIA, IIIB	21	MOPP × 3, radiation, MOPP × 3	81	67.5 at 30 mos
	23	ABVD × 3, radiation, ABVD × 3	91	94.5 at 30 mos
IV	17	MOPP × 12 cycles	53	65.0 at 24 mos
	22	MOPP alternating with ABVD × 12 cycles	55	100 at 24 mos

active combinations is difficult to analyze because of patient heterogeneity, inadequate numbers for comparison, and institutional variability.

Acute Nonlymphocytic Leukemia

A recent comprehensive analysis of randomized trials in this disease suggested that daunorubicin used alone was as active in remission induction as the "best" combinations containing cytosine arabinoside, thiopurines, vincristine, and prednisone (18). Sequential studies by the CALGB determined that intensive cytosine arabinoside infusions and daunorubicin produced the best remission rates seen by that group, including less intensive regimens using the same two-drug combination (19). This has led the group to regard the 7-day infusion of cytosine arabinoside together with daunorubicin given by iv push on Days 1–3 as their best regimen, now titled "7 + 3." Concomitantly, the SWOG combined Adriamycin with aggressive cytosine arabinoside infusion, vincristine, and prednisone for their best remission induction and survival to date (20). A pilot study of the "7 + 3" regimen substituting Adriamycin for daunorubicin gave similar early results (21), was confirmed in a small number of randomized patients (22), and is now the subject of a group-wide investigation by the CALGB.

Hodgkin's Disease

The mechlorethamine, vincristine, prednisone, and procarbazine (MOPP) program developed by DeVita et al (23) represented a major advance in cancer treatment. Numerous substitutions including nitrosoureas for mechlorethamine and vinblastine for vincristine failed to improve the therapeutic efficacy or duration of response (24). In 1975, Bonadonna et al (48) reported a combination of Adriamycin, bleomycin, vinblastine, and DTIC (ABVD). ABVD proved as efficacious as MOPP in previously untreated patients and a lack of cross-resistance was noted for both combinations on crossover after relapse. In expanded studies ABVD appears to be at least equivalent to MOPP in combined modality therapy (26) and may prolong remission duration when given in a cyclic alternating schedule with MOPP in stage IV disease (25).

Non-Hodgkin's Lymphoma

Adriamycin is unquestionably highly active yet its routine use in combination chemotherapy programs raises two general questions.

1. Do nodular lymphomas require intensive combination therapy for prolonged control? Does Adriamycin add to the rate and duration of response?
2. What is the role of Adriamycin in combination therapy for diffuse histiocytic lymphoma and other unfavorable histologies?

Although randomized trials are few, a few general observations are appropriate.

Nodular Histology

The SWOG has identified a highly active combination of cyclophosphamide, Adriamycin, vincristine, and prednisone (CHOP) that has produced an 83% CR rate in nodular poorly differentiated lymphocytic lymphoma (27). A comparison with the same combination *omitting* Adriamycin (HOP) gave a 66% CR rate. However, lymphocytic histology overrode nodularity since diffuse lymphocytic lymphoma also demonstrated superiority for CHOP. Thus, this study stressed the key role of cyclophosphamide in lymphocytic histology and did not totally define the role of Adriamycin.

The current study of the SWOG suggests similar activity for cyclophosphamide, vincristine, prednisone, and bleomycin (COP + bleomycin) compared to CHOP with bacillus Calmette Guérin (BCG) or bleomycin (28). Response rates are lower (± 58%) in all three combinations because of systemic restaging. When fully evaluated, this study will be more definitive with regard to the value of Adriamycin.

Another randomized trial incorporating Adriamycin into one arm is not completely evaluable because of small numbers of patients, but suggests little initial improvement in response rates for Adriamycin combinations (29).

Thus, in summary, the role of Adriamycin in nodular lymphomas is tentative and longer followup is necessary. It may be that survival will not be improved over that produced by more conservative management (30).

Diffuse Lymphomas With Particular Reference to Diffuse Histiocytic Histology

The National Cancer Institute experience suggests that aggressive therapy with cyclophosphamide-MOPP or bleomycin, Adriamycin, cyclophosphamide, vincristine, and prednisone may result in ± 45% CR rates in diffuse histiocytic lymphoma and that 74% of these CRs are extraordinarily durable (31). Several regimens with or without Adriamycin are capable of producing this type of response (32). It is unlikely that many randomized trials will be done in view of the high activity of Adriamycin-containing combinations. Three trials currently in progress are of particular interest.

1. The Milan trial compares COP to Adriamycin, bleomycin, and prednisone (ABP) (29). In the subset of diffuse histiocytic lymphoma nearly identical remission rates are achieved (33% CR rate for COP versus 43% CR rate for ABP), but remission duration is longer (24 months for ABP versus 9 months for COP), although not statistically significantly so.

2. The SWOG trial compares CHOP + BCG to CHOP + bleomycin to COP + bleomycin (28). Although the response rates are similar, remission duration may be superior for CHOP + BCG. This study awaits definitive followup and analysis and should aid in defining the role of Adriamycin. Their previous CHOP-HOP study (27) suggested a higher CR rate in both diffuse and nodular histiocytic histology for HOP with its higher dose of Adriamycin.

3. The ECOG is performing an interesting study on diffuse non-Hodgkin's lymphoma comparing Adriamycin, two schedules of bleomycin, or a nitrosourea (BCNU) added to the basic COP program (33). The Adriamycin arm (COPA) and the sequential bleomycin arm (CPOB) where cyclophosphamide and prednisone are given on Days 1 and 1–5 respectively and low-dose bleomycin and vincristine are given on Day 15 of each cycle showed CR rates of 55% and 59% compared to a 31% CR rate for the simultaneous bleomycin arm (31% for COPB) and a 35% CR rate for the BCNU arm (BCOP). These are early results and remission duration and survival information are incomplete.

Thus, it can be said that Adriamycin is an important part of modern diffuse lymphoma treatment but we must await definitive results. Furthermore, the presence of other active agents (eg, cyclophosphamide and bleomycin) compounds the difficulty of evaluating any single component of a combination.

Soft Tissue Sarcomas

A series of studies by the SWOG has defined Adriamycin as active with increasing response rates and durations when combined with DTIC; cyclophosphamide, vincristine, and DTIC; and cyclophosphamide, vincristine, and actinomycin D (34). Adriamycin remains unquestioned as a necessary ingredient. The ECOG recently completed a randomized study of Adriamycin alone versus Adriamycin, cyclophosphamide, and vincristine versus actinomycin D, cyclophosphamide, and vincristine (14). Adriamycin alone was most active (28% CR + partial response [PR] rate) compared to the Adriamycin combination (13% CR + PR rate) and the actinomycin D combination (5% CR + PR rate). The authors recommended that Adriamycin be used at full dose (70 mg/m^2 iv q3weeks in this study) for maximum benefit, further supporting observations of a dose-response curve in soft tissue sarcomas (12). Further comparative trials are ongoing and will define the optimum Adriamycin combinations in terms of rates and durations of response.

Breast Cancer

A recent comprehensive review of the therapy of advanced breast cancer emphasized that Adriamycin combinations are extremely active but cautioned that durations of response and survival are probably not improved (35). Since the publication of this paper, comparative trials attempting to define the role of Adriamycin have been reported and are summarized in table 4.

These studies suggest a small, finite level of improvement in rates and durations of response. The Southeastern Cancer Study Group studied an intermittent cyclophosphamide, methotrexate, 5-FU, vincristine, and prednisone (CMFVP) combination which seems to have less activity than the cyclophosphamide, methotrexate, and 5-FU (CMF) regimen of Bull or the CALGB regimens reported by Tormey; hence, the magnitude of the difference is greater even with a lower response rate to cyclophosphamide, Adriamycin, and 5-FU (CAF). The Mayo Clinic (Rochester, Minn) trial reported by Ahmann showed a lower response rate with Adriamycin-melphalan (ADR-LPAM) compared to cyclophosphamide, 5-FU, and prednisone (CFP). The combination of ADR-LPAM may not be equivalent to the more aggressive CAF and cyclophosphamide, Adriamycin, 5-FU, vincristine, and prednisone (CAFVP) regimens, and the CFP and CAFVP regimens also contain prednisone. The fixed crossover regimen received a conservative interpretation by the investigators which cannot be regarded as definitive at this early date. A fixed crossover study of CMF plus prednisone alternating with Adriamycin-vincristine is in progress in the ECOG based on the study by De Lena et al (40) demonstrating equivalent efficacy and lack of cross resistance for these two combinations.

TABLE 4.—Comparative trials of Adriamycin combinations in advanced breast cancer

Investigator (ref No.)	Treatment programs	% CR	% CR + PR	Comments
Bull et al (36)	CMF	7.5	62	Survival, 17 mos median
	CAF	18.0	82	Survival, 27.2 mos median ($P = 0.13$)
Smalley et al (37)	CMFVP, intermittent	4.0	37	Duration of response, 22 wks median
	CAF	16.0	64	Duration of response, 32 wks median ($P = 0.02$)
Tormey et al (38)	CMFVP, intensive with 12-wk induction	9.0	65	% CR + PR = % CR + PR + "improved"; longer duration of response for Adriamycin combination
	CMFVP, monthly cycles	11.0	65	
	CAFVP, monthly cycles	16.0	76	
Ahmann et al (39)	CFP	—	31 at 10 wks	Re-randomized at 10 wks; 11 PRs among 20 patients receiving CFP (60%), 11 PRs among 22 patients receiving ADR-LPAM (50%), and 29 PRs among 41 patients in fixed crossover study (70%); progression-free interval similar for the 3 programs
	ADR-LPAM	—	34 at 10 wks	
	2 cycles of CFP alternating with 2 cycles of ADR-LPAM if improved or stable at 10 wks	—	—	

POTENTIAL FOR ADRIAMYCIN IN COMBINED MODALITY THERAPY

In spite of the potential for cardiotoxicity, short-term Adriamycin therapy is under study in the adjuvant setting in a variety of lethal neoplasms.

Osteogenic sarcoma is an area of great interest. The CALGB has reported that Adriamycin in full doses improves the 5-year survival after amputation to approximately 50% if the protocol is strictly followed, and produces only borderline effect if excessive dose reductions are used (13) or surgery is suboptimal. Two of 68 patients who completed the protocol dose of 540 mg/m^2 of Adriamycin developed fatal CHF showing the necessity to push therapy beyond totally safe limits.

Attempts to reduce the total dose of Adriamycin to 450 mg/m^2 by alternating it with high-dose methotrexate are in progress at the Sidney Farber Cancer Institute, Boston, Mass (41). In this trial, 16 of 22 patients are free of metastases with followups ranging from 18+ to 36+ months.

Even greater dose reductions are apparent in the multidrug SWOG programs (COMPADRI I, II, and III) (42), with 42%–55% of patients disease-free at 2 years.

It has not been established which of these programs are superior at this time or whether they will prove superior to the original high-dose methotrexate and vincristine program developed at the Sidney Farber Cancer Institute (41). Furthermore, the Mayo Clinic has noted a progressive improvement in disease-free survival in patients treated with surgery alone (43). This may be attributed to patient selection and staging or a changing natural history.

The success of adjuvant chemotherapy in operable breast cancer led the M. D. Anderson Hospital and Tumor Institute group (Houston, Tex) to use an Adriamycin-based regimen plus BCG in stage II–III breast cancer after primary treatment with mastectomy and postoperative radiotherapy (44). The results of 5-FU, Adriamycin, and cyclophosphamide plus BCG at 2 years were compared to historical control patients with favorable results. The total dose of Adriamycin was limited to 320 mg/m^2 and no cardiotoxicity was encountered. This may be a very important study if the preliminary results of benefit in both pre- and post-menopausal women persist. However, length of followup must be equivalent to the more mature CMF trial before conclusions can be reached (45).

CLINICAL INVESTIGATIONS IN OTHER SOLID TUMORS

This review has concentrated on a selected group of neoplasms where anthracycline therapy has made significant contributions. Of the numerous responsive tumors shown in table 1, intense interest in Adriamycin therapy is apparent in lung, gastric, ovarian, endometrial, bladder, and prostate carcinomas and hepatomas. Space will not permit documentation of the many trials in progress. However, the utility of non-cross-resistant combinations in small cell lung cancer is being tested in the National Cancer Institute-Veterans' Administration Medical Oncology Branch (Washington, DC) where CCNU, cyclophosphamide, and methotrexate is alternated with vincristine, Adriamycin, and procarbazine (46). A 56% CR rate and a 92% overall response rate have been observed in limited-disease patients. Long-term followup is awaited with interest. The combination of Adriamycin and DDP is active in a variety of solid tumors and is especially promising in ovarian carcinoma (47). It is hoped that the full promise of Adriamycin will be realized with these many trials.

CONCLUSIONS

As in any review, the conclusions are mixed. The anthracyclines have been demonstrated to occupy an important place in cancer chemotherapy. However, defining their exact role is especially difficult when they are used in combination with other active agents.

In acute leukemia the anthracyclines are established as a major therapeutic tool. The primacy of daunorubicin is currently being challenged by the substitution of Adriamycin in active combination programs.

At least one Adriamycin combination program appears not to be cross-resistant with MOPP and is of equal efficacy in Hodgkin's disease. Alternating MOPP with ABVD is feasible and may be the only successful application of improved therapeutic results with alternative combination chemotherapy.

The final role of Adriamycin in non-Hodgkin's lymphoma will not be clarified until the necessary followup has ensued. This is especially true for lymphocytic histologic subtypes.

The best example of a dose-response curve is seen in soft tissue sarcomas where Adriamycin is the major active agent. The optimum combination program is yet to be defined.

Adriamycin combinations are modestly superior in advanced breast cancer; significant prolongation of remission and survival has not been noted to date. Alternating combinations remain an unsolved question in this disease.

Adjuvant activity in osteogenic sarcoma is highly likely. Years of further investigation may be necessary to define the adjuvant role of Adriamycin in this rare tumor. If, indeed, the natural history of osteogenic sarcoma is changing, controlled studies may be critical to settle the role of adjuvant therapy in general.

Notable activity is apparent in lung, stomach, liver, and genitourinary tract cancers. Conclusions cannot be made presently since investigations are not yet definitive.

The prevention of cumulative cardiotoxicity remains a major goal and cardiotoxicity continues to limit the usefulness of the anthracyclines. Dose-limitation and scheduling currently are of interest but only development of noncardiotoxic analogs and/or prevention of cardiac damage by ancillary therapy will provide enhanced safety so maximum benefit can be achieved.

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Anthracycline Antibiotic Pharmacology and Metabolism

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SUMMARY

The anthracycline antibiotics show evidence of numerous interactions with cellular components and participation in several metabolic pathways. Small structural changes in the antibiotic molecule can produce major changes in intracellular localization and interaction. To date, we find that all of the anthracycline antibiotics form free radical intermediates through the catalysis of microsomal and nuclear electron-transport machinery. NADPH cytochrome P-450 reductase catalyzes the free radical formation. The free radical intermediates ("site-specific free radicals") may be causative of both toxicity and pharmaceutic action. The free radical intermediates degrade nonenzymatically to 7-deoxyaglycone metabolites which prove the existence of the free radical in vivo.

[Cancer Treat Rep 63:817–820, 1979]

Since the discovery of the anticancer activities of daunorubicin and Adriamycin, a large number of analogs, derivatives, and complexes of the anthracycline antibiotic family have been isolated, synthesized, and assessed for anticancer activity. Only a few of these agents, however, have been used in clinical trials. Any attempt to review the pharmacology and metabolism of even those few agents would represent a major enterprise. Rather, general principles of anthracycline antibiotic pharmacology will be described along with new findings concerning their biotransformation. The principal subjects of pharmacologic investigation have been daunorubicin, Adriamycin, AD-32, carminomycin, aclacinomycin A, and rubidazone.

Semisynthetic and natural derivatives of the basic anthracycline structure that are currently of clinical and research interest (fig 1) include the following: (a) *modifications of the A ring* (4-hydroxydaunorubicin [carminomycin] and 4-demethoxyl compounds); (b) *modifications of the D ring* (carbonyl substitutions, eg, benzoyl hydrazone of daunorubicin [rubidazone]; esterification of Adriamycin, eg, octanoyl ester and valeryl ester [one modification of AD-32]); (c) *other ring modifications* (iminodaunorubicin, first synthetic alteration of quinone-hydroquinone system); and (d) *sugar modifications* (amino substitutions, eg, *N*-trifluoroacetyl [another modification of AD-32], *N*,*N*-dibenzoyl, 4′-epiadriamycin, and 4′-epidaunorubicin).

In addition to these chemical modifications, other naturally occurring anthracycline-type compounds and their derivatives such as nogalamycin, aclacinomycin A, the pyromycins, etc, have been isolated and found to have activity against animal and human tumors.

The multipotential character of the anthracyclines has been emphasized in an earlier discussion of Adriamycin pharmacology (1). Much of the basic work on these agents stressed their interaction with DNA, their intercalative binding to double helical DNA, and the resultant biochemical consequences of this binding (2). The interaction with DNA is critical to their mechanism of action, but their interactions with other tissue components are also critical to their pharmacology and possibly their anticancer selectivity.

Investigators are reporting various interactions between anthracycline antibiotics and tissue components. Menozzi and Arcamone (3) examined binding between sulfated mucopolysaccharide and Adriamycin. Na and Timasheff (4) described binding of tubulin and Adriamycin. It is also clear from metabolism studies that the anthracyclines interact with cytoplasmic as well as microsomal proteins, since enzymes specific to those cellular regions metabolize anthracyclines.

Most anthracyclines must be administered iv to exhibit their activity although a few (eg, carminomycin and acla-

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cinomycin A) have activity on oral administration. On iv administration, the anthracyclines are rapidly removed from the vascular and extracellular compartments into the intracellular compartment, exhibiting large volumes of distribution. The elimination of drug occurs slowly as the plasma drug concentration shows a prolonged β phase. When the anthracyclines are made more lipophilic as in the case of AD-32, their sequestration by liver is very high (5). Anthracyclines bound to hydrophilic macromolecules such as DNA show an intravascular half-life dependent on the macromolecule. Thus, the half-life is increased resulting in higher prolonged plasma concentrations (6).

Anthracyclines enter cells very rapidly and concentrate intracellularly manifold over the plasma concentration. Some are bound to nuclear DNA which serves as an intracellular binding reservoir, thereby enhancing cell uptake and cell:plasma ratio. This is true for such agents as Adriamycin and daunorubicin. However, some anthracyclines such as AD-32, aclacinomycin A, and carminomycin are not "nucleophilic" but are concentrated in the cytoplasm. Despite this difference in intracellular localization, the cytoplasmotropic agents are still taken up avidly by cells (Egorin MJ, et al, unpublished observations).

Tissue distribution of anthracyclines has not been stud-

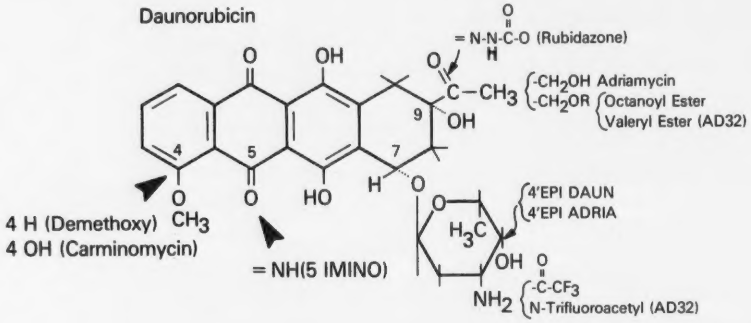
Daunorubicin
= N-N-C-O (Rubidazone)
-CH2OH Adriamycin
-CH2OR
Octanoyl Ester
Valeryl Ester (AD32)
4 H (Demethoxy)
4 OH (Carminomycin)
= NH(5 IMINO)
4'EPI DAUN
4'EPI ADRIA
-C-CF3
N-Trifluoroacetyl (AD32)
4
5
7
9

FIGURE 1.—Structure of first clinically useful anthracycline anticancer antibiotic, daunorubicin, and structural modifications of important analogs.

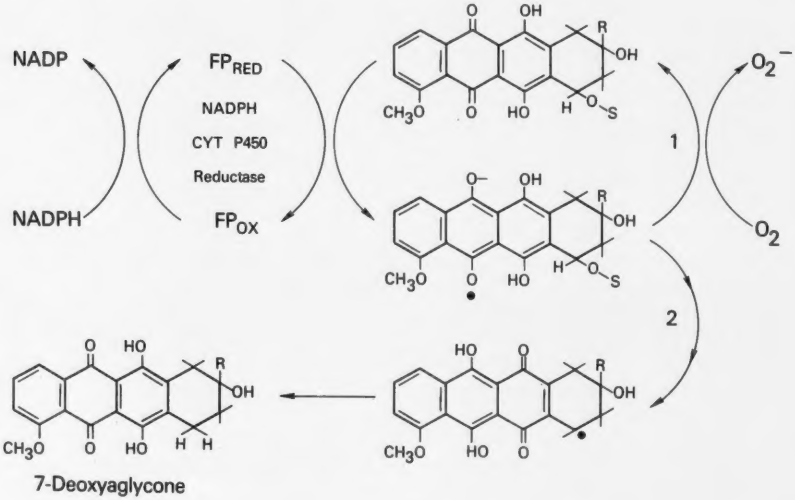
NADP
NADPH
FPRED
NADPH
CYT P450
Reductase
FPOX
O2−
O2
1
2
R
OH
S
7-Deoxyaglycone

FIGURE 2.—Mechanism for microsomal (NADPH cytochrome P-450 reductase) catalysis of anthracycline free radical formation with subsequent nonenzymatic rearrangement to 7-deoxyaglycone product. FP_{RED} = flavoprotein reduced; FP_{OX} = flavoprotein oxidized.

ied adequately, but for the drugs studied the highest concentrations occur in kidney, liver, spleen, stomach, and lymph nodes (7,8).

For those anthracyclines studied, excretion is primarily via the liver and biliary system. Only small amounts (5%–15%) of the agents are excreted via the kidney and urine (7,9). For this reason, our original guidelines proposed caution, and dosage reduction in patients with liver dysfunction will probably be applicable to all anthracyclines used clinically (10).

Disposition studies with several anthracyclines in animals and man show an incomplete recovery of the administered dose (8,9). By fluorescence measurement in man, only about 65% of the administered dose is recovered (9). Studies with radioactive compounds either tritiated by Wilzbach technique or labeled with ^{14}C indicated higher, but still incomplete, recoveries. About 80% of the administered radioactivity of ^{14}C-labeled Adriamycin is recovered from rats (8). Of this, approximately 7% is in expired CO_2 and 65% is in feces. This incomplete recovery is probably an important message concerning the metabolic fate of the agents.

All of the anthracycline antibiotics and their semisynthetic analogs show evidence of extensive metabolism in mammals. The metabolic patterns and preferences for pathways seem altered with some analogs, but the metabolism follows the basic pathways previously described (11,12).

Anthracyclines which contain a reducible carbonyl in their C-9 side chains (eg, Adriamycin, daunorubicin, carminomycin, AD-32, etc) yield an alcohol metabolite which is a product of the intracellular cytoplasmic aldo-keto-reductases. Aldo-ketoreductases are a family of enzymes, quite active in the reduction of xenobiotic aldehydes and ketones (13). The products of this enzymatic reaction with daunorubicin and Adriamycin, daunorubicinol and adriamycinol, are prominent metabolites retaining the cytotoxic action of the parent molecules. The reduction of the carbonyl group does not interfere with the action of these metabolites in binding to cellular DNA or in inhibiting nucleic acid metabolism. The most apparent effect of this carbonyl reduction is to alter the lipid solubility of the antibiotic molecule thereby altering the binding to lipid-rich membranes and the membrane transit of the metabolite.

One of the most significant metabolic alterations to anthracycline antibiotics occurs at the microsomal level in an enzymatic reaction called the reductive glycosidase (fig 2). Most of the anthracyclines studied undergo this metabolic conversion in vivo as proven by the existence of deoxyaglycone conjugates excreted in urine and bile. In our studies of this biotransformation, we observed that the reaction was inhibited by oxygen, required NADPH, and was primarily microsomal in location, although nuclear membranes also catalyze the event (14). We have purified and isolated the enzyme capable of this conversion and identified it as NADPH cytochrome P-450 reductase (15). Similarly, Oki et al (16) have reported the reductive cleavage of aclacinomycin A and other anthracyclines by NADPH cytochrome P-450 reductase.

The anthracycline antibiotics participate in an important single electron transport in microsomes. We have shown that in the microsomal system, a single electron transfer from NADPH to a microsomal flavoprotein and subsequently to the anthracycline antibiotic results in the formation of a semiquinone form of the anthracycline, a free radical form (17). NADPH cytochrome P-450 reductase isolated from microsomes catalyzes the formation of the free radical form of the anthracycline (18).

When the semiquinone form of the drug is produced, it readily transfers its single electron to oxygen to form superoxide and thereby cycles back to the stable quinone form (fig 2 [1]). If oxygen becomes limiting, the semiquinone form apparently cannot donate its electron rapidly enough to other electron acceptors. In the semiquinone state, the anthracycline undergoes a rearrangement (fig 2 [2]) whereby the glycosidic bond, by virtue of the oxygen-containing ether linkage, is sufficiently electron-withdrawing to attract the unpaired electron and to cleave the glycosidic bond. Hydrogen abstraction replaces the sugar, completing the reaction and yielding the product 7-deoxyaglycone. This mechanism requires that the first step be the enzymatic formation of the free radical semiquinone which is very reactive. If no electron acceptor is available, the unstable semiquinone radical rearranges and the glycosidic bond cleaves spontaneously and nonenzymatically. The chemical basis for this type of reductive transformation at the C-7 position has been shown previously by Arcamone et al (19) in palladium-catalyzed reduction to the deoxyaglycones and this has been confirmed in our laboratory. We have also detected a free radical intermediate in this chemical transformation (unpublished observations).

Since the reductive cleavage is directly the result of a free radical intermediate, the existence of deoxyaglycones as free aglycones or conjugates in bile, urine, and tissues demonstrates that the anthracycline antibiotics exist in a free radical state in vivo. Since we have identified deoxyaglycone metabolites in most tissues including the heart, liver, and kidney (9,20), and since the microsomes from these tissues carry out the reductive cleavage (14) and contain NADPH cytochrome P-450 reductase, the free radical state of the anthracyclines is a common metabolic occurrence. The free radical state of the anthracyclines is probably directly related to both the anticancer activity of the agents (21) and their cardiotoxicity (22).

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Biochemical Parameters of Growth Inhibition of Human Leukemia Cells by Antitumor Anthracycline Agents[1]

Herbert S. Schwartz* and Peter M. Kanter[2]

SUMMARY

Ten antitumor anthracycline derivatives used in this study inhibit growth of a human leukemia cell line over a range of potencies exceeding four orders of magnitude. The agents vary from each other at C-4 and C-13, with stereochemical differences at C-4′ and substitutions at C-14 and N. Drug retention, DNA damage, and inhibition of DNA synthesis are parameters used to express potency of the agents in a mathematic model. Estimates of DNA damage are sufficient, however, to describe growth inhibition by a single agent (4-demethoxydaunorubicin). It may be possible now to attempt to extend the model to predict therapeutic responses to a number of anthracyclines, thereby enhancing the clinical utility of this important class of agents.

[Cancer Treat Rep 63:821–825, 1979]

Antitumor anthracyclines (fig 1) may be classed generally as DNA-intercalative compounds, while those with *N*-acyl-substituted groups appear to associate weakly, if at all, with DNA (1,2). Besides this apparent target, a number of anthracyclines associate with membranes, binding ionically to proteins and reacting with negatively charged phospholipids (3–7). The recent studies by Handa and Sato (8) and by Bachur et al (9) demonstrate that Adriamycin and daunorubicin as well as other quinone-containing agents may be activated to free radical forms by microsomal enzymes. From these observations, it seems likely that DNA damage, encountered in sensitive cells treated with the antitumor anthracyclines, may result from metabolic activation which permits the agents to bind covalently to DNA and thereby generate lesions within the helix.

The physical effects of anthracycline intercalation and ionic binding, and the assumed covalent attachment of activated forms of the agents, alter the DNA target and affect its template functions (1,2). Those agents that bind reversibly to DNA by intercalation may, in addition, be considered as a depot form, having a $K_d < 1 \times 10^{-6}$ M. From such a depot, the agents are likely to be released slowly and then transformed to radicals and less active forms. Because the drugs can be stored in this fashion, we suspect that intercalative binding accounts for some of the delayed actions of the anthracyclines in sensitive cells: limited DNA repair, inhibition of DNA and RNA synthesis, and lethal actions in all phases of the mitotic cycle. To the actions stemming from intercalation, however, must be added the effects of DNA damage, particularly when the lesions occur at closely adjacent sites in both strands. Damage of this nature may result in double-strand breaks which are thought to be irreparable and probably lethal, but even single-strand lesions may be lethal if repair mechanisms are blocked by the intercalated ligand.

It is likely that a multitude of events may contribute to the biologic effects of the anthracyclines. To enumerate just a few, selective growth inhibition and cell death may depend upon such factors as drug uptake and retention, activation and inactivation biotransformations, and availability of and affinity for target sites, with modulation by functional recovery because of target turnover, replacement, or repair systems. There have been a number of attempts to select determinant factors that correlate with potency of the anthracyclines; these parameters include lipophilicity, drug uptake and metabolism, inhibi-

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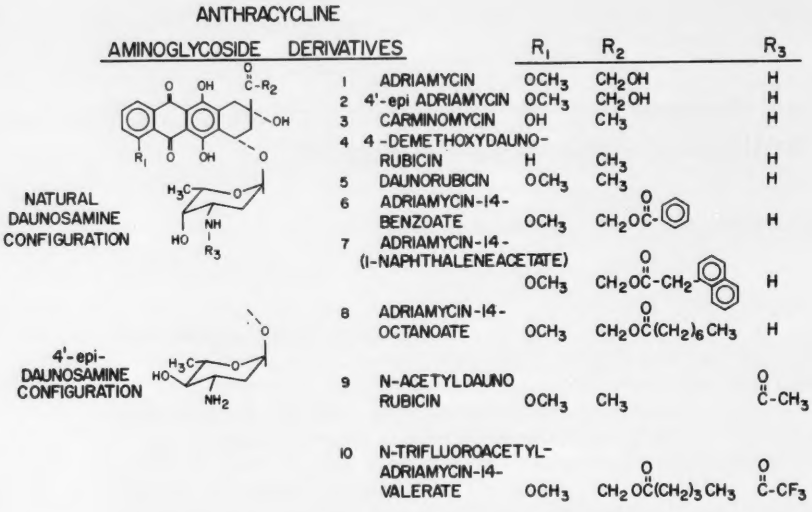
ANTHRACYCLINE
AMINOGLYCOSIDE DERIVATIVES
NATURAL DAUNOSAMINE CONFIGURATION
4'-epi-DAUNOSAMINE CONFIGURATION
R_1
R_2
R_3

	ANTHRACYCLINE AMINOGLYCOSIDE DERIVATIVES	R_1	R_2	R_3
1	ADRIAMYCIN	OCH_3	CH_2OH	H
2	4'-epi ADRIAMYCIN	OCH_3	CH_2OH	H
3	CARMINOMYCIN	OH	CH_3	H
4	4-DEMETHOXYDAUNORUBICIN	H	CH_3	H
5	DAUNORUBICIN	OCH_3	CH_3	H
6	ADRIAMYCIN-14-BENZOATE	OCH_3	$CH_2O\overset{\overset{O}{\|}}{C}$-	H
7	ADRIAMYCIN-14-(I-NAPHTHALENEACETATE)	OCH_3	$CH_2O\overset{\overset{O}{\|}}{C}-CH_2$-	H
8	ADRIAMYCIN-14-OCTANOATE	OCH_3	$CH_2O\overset{\overset{O}{\|}}{C}(CH_2)_6CH_3$	H
9	N-ACETYLDAUNORUBICIN	OCH_3	CH_3	$\overset{\overset{O}{\|}}{C}-CH_3$
10	N-TRIFLUOROACETYL-ADRIAMYCIN-14-VALERATE	OCH_3	$CH_2O\overset{\overset{O}{\|}}{C}(CH_2)_3CH_3$	$\overset{\overset{O}{\|}}{C}-CF_3$

FIGURE 1.—Chemical structures of anthracycline derivatives.

tion of DNA and RNA synthesis, DNA affinity, and effects of intercalation (10–13). There have been no prior reports, however, that incorporate DNA damage into a model of intercalative drug action.

To carry out such a study, human lymphoblastic leukemia cells (CCRF-CEM) were incubated for 2 hours with the anthracycline derivatives shown in figure 1. Each of the agents has a different initial rate of uptake but they generally approach plateau levels by 2 hours at the concentrations used. The cells were then washed and reincubated in drug-free medium. At consecutive times, DNA damage was assayed by a modified Rydberg procedure (14); the results are expressed as breaks (n) per DNA unwinding unit (Mn_o, calculated as 4×10^8 daltons for CCRF-CEM cells). Because the method uses alkaline conditions (0.05 N NaOH), n does not distinguish between frank strand breaks and alkali-labile regions but only indicates the number of sites at which DNA unwinds in alkali. Each site (corrected for those initially present) represents a point of damage in the unwinding unit.

Some of the DNA-damaging actions of three anthracyclines may be compared in figure 2 after CCRF-CEM cells are exposed for 2 hours to Adriamycin, *N*-trifluoroacetyladriamycin-14-valerate (AD-32), and *N*-acetyldaunorubicin (10 μg/ml). The results show delayed DNA damage appearing in washed cells after treatment with Adriamycin. The number of lesions then increases and reaches a plateau by 6–8 hours after the initial incubation. Similar kinetics of DNA damage are observed with other

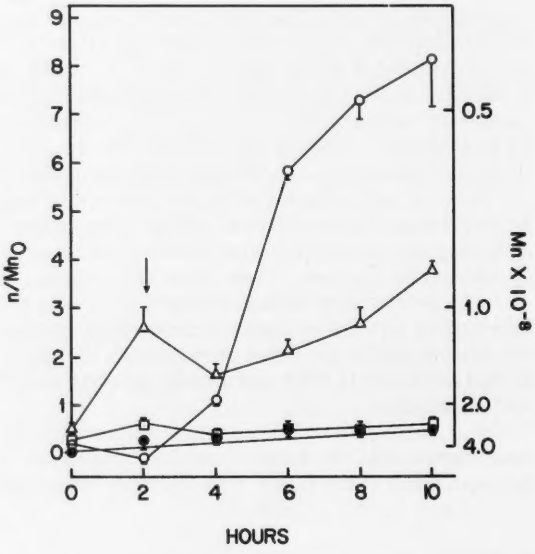

FIGURE 2.—DNA damage (n) per alkaline unwinding unit (Mn_o) by anthracycline derivatives in CCRF-CEM cells. DNA damage (n) was assayed in CCRF-CEM cells incubated for 2 hrs with 10 μg/ml each of Adriamycin (○), AD-32 (△), and *N*-acetyldaunorubicin (□), and without added drug (●). Cells were washed (arrow) and reincubated in drug-free medium, and damage is estimated at times indicated. Vertical lines indicate standard deviation. DNA damage and DNA unwinding unit are described in the text.

unsubstituted anthracyclines (ie, compounds ***1–5*** in fig 1) with plateau level damage proportional to the initial drug concentration (not shown). In contrast to the results with Adriamycin, AD-32 induces earlier DNA damage but with partial repair after the cells are washed; thereafter, damage again increases. *N*-Acetyldaunorubicin generates only a barely detectable amount of damage which is at the limits of sensitivity (0.1 n/Mn_o) in CCRF-CEM cells. The partial repair with AD-32 has been seen repeatedly in other experiments and (with the possible exception of *N*-acetyldaunorubicin) has not been observed with other anthracyclines in our experiments. It seems likely that partial repair may be due to the low affinity of AD-32 (or the de-esterified metabolite) for DNA. Estimations of effectiveness (ie, intracellular drug molecules per DNA lesion) indicate that Adriamycin is two or three times more efficient than AD-32 as a DNA-damaging agent. These results suggest that a target-oriented action due to DNA intercalation may provide for more efficient utilization of an agent such as Adriamycin in generating DNA lesions. Intercalation also appears to delay the onset of damage, although it may prolong and perhaps intensify the effect by blocking repair mechanisms and by protecting the agent from cellular inactivation mechanisms.

In a more comprehensive study, we assayed growth, DNA synthesis and damage, and retention of the agents (fig 1) by CCRF-CEM cells incubated under standard conditions. As before, cells were incubated for 2 hours with each agent, all at 1 μg/ml (except compounds ***9*** and ***10***, the *N*-acylated derivatives, which were used at 10 μg/ml). The cells were then washed and reincubated (6 hours) in drug-free medium. At this time DNA damage (n), inhibition of DNA synthesis (I), and drug retention (r) were measured. Drug retention (nmols per 1 × 10^6 cells) was estimated by fluorescence techniques after $AgNO_3$-isoamyl alcohol extraction, using parent compounds as internal standards. Tritiated thymidine incorporation (50 microcuries/nmol; 1 curie/ml) as cold acid-insoluble radioactivity was measured for a 1-hour period (uptake was unaffected by the agents); inhibition (I) was calculated as 1 – (treated incorporation/control incorporation). Relative lipophilicity (R_f) was determined by silica-gel G thin-layer chromatography with $CHCl_3$:methanol:0.01 M tris, pH 7.4 (80:20:3); values given are proportional to partition coefficients between sodium phosphate buffer (0.01 M, pH 7.4) and isoamyl alcohol. The results are shown in table 1 where the agents are listed in order of increasing relative lipophilicity. The values of r shown in parentheses are intracellular concentrations of Adriamycin formed from compounds ***6, 7,*** and ***8*** (measured after incubations by thin-layer chromatography separation techniques).

To measure cell growth, cells (1 × 10^6, 4 ml) in logarithmic growth were incubated for 2 hours with each derivative (five to seven concentrations in twofold increments), washed, and reincubated in fresh medium (RPMI-1640, 10% fetal calf serum) for 50 hours. Growth curves shown in figure 3 indicate that the anthracycline derivatives (numbered as shown in table 1) inhibit growth over a 10,000-fold range of concentrations. Table 1 shows initial concentrations that inhibit growth by 37% (ID37).

In attempting to develop a mathematic model to describe potency of the agents in terms of biochemical parameters, we assume that DNA damage (n) and inhibition of DNA synthesis (I) both contribute to biologic activity. The specific activity (*a*) is then defined as I × n per unit of retained drug (r):

$$a = \frac{I \times n}{r}. \qquad [1]$$

The values of *a* shown in figure 4 are graphed against R_f and compared with the potency (P; defined as 1/ID37) of

TABLE 1.—Growth inhibition, uptake, and DNA-related effects of anthracycline derivatives with CCRF-CEM cells*

Compound	R_f	ID37 (M × 10^6)	r (nmols/10^6 cells)	I	n
1, Adriamycin	0.16	0.18	0.16	0.42	0.52
2, 4′-epiadriamycin	0.19	0.13	0.24	0.68	0.85
3, carminomycin	0.21	0.0078	0.89	> 0.99	9.8
4, 4-demethoxydaunorubicin	0.22	0.014	0.58	> 0.99	6.7
5, daunorubicin	0.24	0.038	0.43	0.98	2.2
6, adriamycin-14-benzoate	0.29	0.14	1.34 (0.067)	0.43	0.27
7, adriamycin-14-(1-naphthaleneacetate)	0.32	0.25	1.64 (0.115)	0.14	1.5
8, adriamycin-14-octanoate	0.34	0.036	1.03 (0.71)	0.79	0.95
9, *N*-acetyldaunorubicin	0.68	10	0.12	0.26	0.10
10, AD-32	0.72	1.0	1.70	0.77	1.5

**See* text for explanation of symbols.

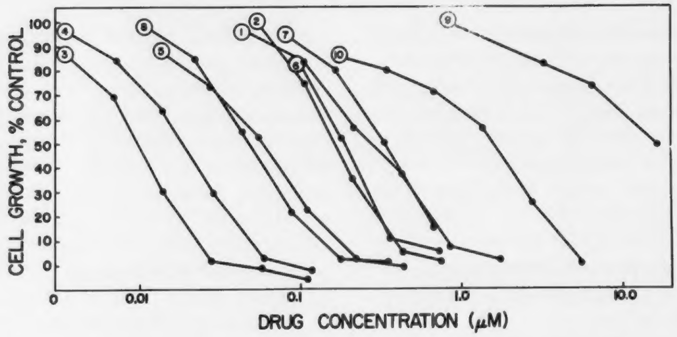

FIGURE 3.—Inhibition of growth by anthracycline derivatives. Each agent is identified by the number shown in fig 1 and table 1.

each agent with CCRF-CEM cells in the assay. The maximal values of a appear at R_f 0.21–0.22 with carminomycin (*3*) and 4-demethoxydaunorubicin (*4*) which are also the most potent agents in the set. The relationship of a to R_f and the general correlation of a with potency appear to hold reasonably well for the other agents, provided that the values of r for compounds *6* and *7* are taken as Adriamycin formed within the cell (shown in parentheses in table 1). The octanoate (*8*) which is more potent than expected from specific activity, even after correction for conversion to Adriamycin, is an apparent exception and may have determinant actions other than those measured here (13,15).

It is possible to express potency of the agents as a function of their specific activity. By substituting in equation 1:

$$P = f\left(\frac{I \times n}{r}\right) \qquad [2]$$

If ln (I × n/r) is plotted against ln P, then the data suggest that these two quantities are linearly related. This expression can be written as:

$$\ln P = k_1 + k_2 \ln\left(\frac{I \times n}{r}\right). \qquad [3]$$

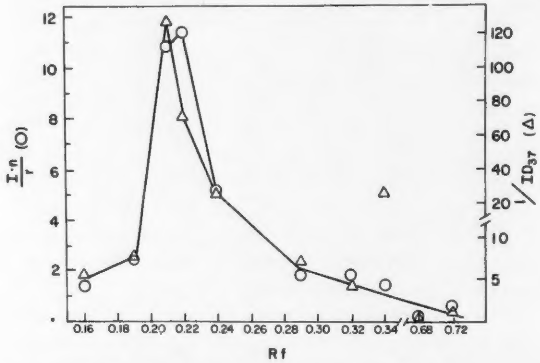

FIGURE 4.—Potency (1/ID37) and specific activity (I × n/r) of the anthracyclines as a function of relative lipophilicity (R_f) (*see* text). Each agent is identified by R_f shown in table 1.

Taking exponentials on both sides of equation 3 and inverting to express the relationship in terms of ID37 (= 1/P), the mathematic model has the general form of an exponential equation:

$$\text{ID37} = K_1\left(\frac{r}{I \times n}\right)^{k_2} \qquad [4]$$

where $K_1 = e^{-k_1}$. The constants K_1 and k_2 are estimated from the least-squares fit of equation 3 and the specific equation for the set of agents (excluding *8*) is:

$$\text{ID37} = 0.77\left(\frac{r}{I \times n}\right)^{1.68} \qquad [5]$$

Figure 5 shows the ID37 values calculated from equation 5 plotted against those in table 1 from growth studies. The regression expressing the relationship was calcu-

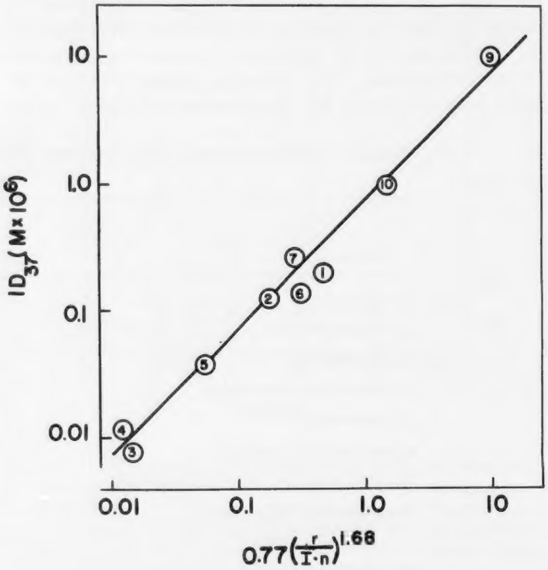

FIGURE 5.—Comparison of inhibition (ID37) from cell growth studies with calculated functions of (I × n/r) (*see* text).

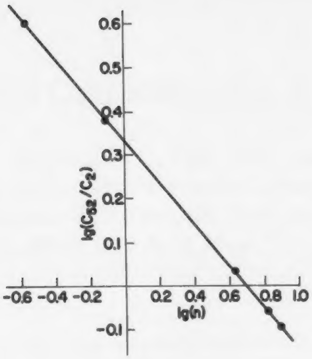

FIGURE 6.—Comparison of cell growth with calculated functions of n (*see* text).

lated by the least-squares method: slope = 1.0; coefficient correlation > 0.99; confidence level (P) of slope < 0.025.

The general model, expressed as equation 4, indicates that I, n, and r are sufficient to express the potency of nine of the agents in the anthracycline set under standardized conditions. Further analysis of the data in table 1 suggests that r and n may be the primary determinants for the unsubstituted derivatives (***1–5***).The esterified (***6*** and ***7***) and *N*-acylated (***9*** and ***10***) compounds also require that I be incorporated into the model to express their potency. The importance of DNA damage as a determinant in a model is demonstrated when the cells are incubated for the same fixed periods of time with varying concentrations of a single agent (4-demethoxydaunorubicin, ***4***). At concentrations where changes in cell numbers are log-linear, the values of n are alone sufficient to suggest a linear relationship with growth (fig 6). Taking growth ratios (cell concentration at 52 hours to that at 2 hours, C_{52}/C_2), the data suggest that:

$$\frac{C_{52}}{C_2} = 2.15/_{n}0.48 \qquad [6]$$

where the change in cell numbers appears to be inversely related to the square root of n when n is > 0.1 (limits of sensitivity). Values of (C_{52}/C_2) calculated from n in equation 6 have been compared to those from growth curves. The least-squares method indicates that the regression has a slope of 1.0, passing through the origin, with a coefficient of correlation > 0.999.

The results described here suggest that DNA damage may be considered as a sufficient biochemical parameter under specified conditions to predict potency of some of the antitumor anthracycline agents. For most of the anthracyclines, however, neither I nor n varies completely independently of the other in proliferative cells and so both parameters would seem to be required. In the special case where potencies of different agents are compared, the drug retention level (r) may be a determinant; when both I and n are dependent variables, all three parameters are necessary to estimate activity of each agent (or active moiety) in the set. Although other important actions (ie, membrane-associated effects) may determine selectivity or drug activation or both, it seems fortunate that so few parameters appear to be critical to the expression of potency for the anthracyclines. Consistent with the observations described above, studies that are currently in progress indicate that in vitro assays of DNA damage with murine and human leukemia cells may be used to predict responses of these cells to therapy in their respective hosts.

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Adriamycin Cardiotoxicity: A Survey of 1273 Patients

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SUMMARY

Valuable information was collected on the medical history and clinical course of 1273 patients entered in clinical trials with Adriamycin (ADR) carried out in 12 European cancer centers. A coded patient form was used for the data collection carried out in each center by a qualified physician following a guideline which was discussed and accepted by all of the participants. The aim of the study was to define the incidence, characteristics, and possible co-factors of the cardiomyopathy (CMP) in patients treated with combination chemotherapy regimens including ADR. The mean total dose of ADR was 268 mg/m^2 (range, 15–1251 mg/m^2), and 5.1% of the patients received a total dose of $>$ 550 mg/m^2. A "definite" ADR-related CMP was observed in 1.7% of the cases; another 3% of the cases were reported as "possible" ADR-CMP since the role played by the drug could not be clearly defined. "Definite" ADR-CMP was fatal in eight patients (0.6%) while "possible" ADR-CMP was fatal in 13 patients (1.0%). Among the possible co-factors examined, the following ones were found to be significantly associated with the occurrence of a "definite" ADR-CMP: (*a*) total dose of ADR; (*b*) vincristine when given both before and concomitantly with ADR; (*c*) bleomycin when given before ADR; and (*d*) radiotherapy to the mediastinum when given concomitantly with ADR. Furthermore, none of 182 patients receiving ADR by slow infusion developed a "definite" ADR-CMP, while 2% of the patients treated by bolus injection did so. The occurrence of a "possible" ADR-CMP was found to be significantly associated with two pre-existing pathologic conditions (electrocardiogram [ECG] abnormalities and hypertension) but not with the treatment-related co-factors for the "definite" ADR-CMP mentioned above. Other variables examined, such as sex, age, cancer type, baseline liver function, and cyclophosphamide treatment, did not seem to influence the risk of ADR-CMP. Data on ECG changes occurring during ADR treatment were also reported and their incidence was found to be strictly related to the frequency of the ECG monitoring.

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The clinical occurrence of delayed cardiomyopathy (CMP) has been well-known ever since the preliminary phase I and II studies of Adriamycin (ADR) were published (1,2). The detailed reports available on this drug-related phenomenon showed that the overall incidence of CMP ranged from 0 to 10%, when different clinical experiences carried out in several institutions were taken into consideration. However, recent reviews on larger series where patients were subjected to strict evaluation have estimated its incidence to be in the range of 1%–2% (3). It is also generally accepted that high doses of ADR (especially > 550 mg/m^2) seem to increase the risk of CMP, causing an incidence of $\geqslant$ 10%–25%.

The aforementioned data pertain to single-drug treatment, and no clearly defined levels of risk for CMP are as yet available with regard to the combination of ADR with various drugs. At present, polychemotherapy appears to be the most frequently applied treatment of several forms of cancer and a definition of the risk of ADR-related CMP after a combination approach is mandatory. The aim of this evaluation was essentially twofold: (*a*) to define the incidence of ADR-related CMP in a large population treated according to phase III combination regimens within qualified cancer centers in Europe, and (*b*) to attempt to define the occurrence and the importance of factors other than the total dose of ADR which are suspected to influence CMP either from data available in the literature or from direct clinical experience.

PATIENTS AND METHODS

The institutions participating in this study were selected on the basis of the availability of an adequate number of patients treated with regimens containing ADR. Data collection had to be performed by a qualified physician. A form was provided for the reporting of extensive information on each patient, namely: age, sex, tumor type, total dose of ADR, regimen route and schedule of ADR, baseline clinical conditions, prior and/or concomitant treatments, electrocardiogram (ECG) (baseline and during treatment), symptoms and signs of possible CMP, followup related to disease and heart function, and data concerning death and postmortem examination.

CMP was recorded according to a definition mutually agreed upon during a previous meeting with all the investigators involved (fig 1). The collected information was examined by a review committee consisting of three independent investigators (C. Praga, G. Beretta, and P. L. Vigo) who requested further details when necessary. The coding of information and the processing were performed with a Univac 1106 system. The results were analyzed by means of appropriate statistical tests. All patients treated between January 1, 1973, and December 31, 1976, were registered, provided that they had been included in a prospective (usually phase III) treatment protocol.

"Definite" ADR-CMP = ADR was considered to have played a definite role in the occurrence of CMP

"Possible" ADR-CMP = the responsibility of ADR in the occurrence of CMP could not be excluded

No ADR-CMP = (a) no signs of CMP were observed, or (b) CMP occurred but ADR was not considered to be responsible

FIGURE 1.—Definitions of ADR-CMP.

RESULTS

Patient Population and Treatment Characteristics

An outline of all case material collected and reviewed as of July 1, 1978, is shown in table 1. Details on patients treated with > 550 mg/m^2 of ADR are reported separately. Of 1273 study patients, seven were found to be unevaluable due to lack of major information concerning

TABLE 1.—Characteristics of evaluable patients

	Total No. of patients	No. of patients (%) who received $\geqslant 550$ mg/m^2 of ADR
Total population	1266	64 (5.1)
Sex		
Males	574	18 (3.1)
Females	692	46 (6.6)
Age in yrs		
< 15	110	2 (1.8)
15–29	150	7 (4.7)
30–44	239	14 (5.9)
45–59	421	25 (5.9)
$\geqslant 60$	338	15 (4.4)
Type of cancer		
Hematologic	322	17 (5.3)
Solid tumor	933	46 (4.9)
ADR treatment		
1st line	937	54 (5.8)
2nd line	322	9 (2.8)
ADR alone	104	8 (7.7)
ADR in combination	1156	55 (4.8)
Liver function*		
Normal	881	47 (5.3)
Abnormal	208	7 (3.4)
Blood pressure*		
$\leqslant 140/90$	1119	55 (4.9)
$> 140/90$	121	8 (6.6)
ECG*		
Normal	732	48 (6.6)
Abnormal	247	12 (4.9)

*Baseline values.

CMP and/or total dose and timing of ADR administration. Of the 1266 evaluable patients, there was a predominance of female patients. While only 110 patients were children, 338 were > 60 years old. Among patients with solid tumors, most had breast or lung carcinoma; soft tissue sarcomas were the next most common tumors. Radiation therapy to the mediastinum (RTm) was associated with ADR therapy in 319 patients (concomitantly with ADR in 61, before ADR in 176, and after ADR in 82). The majority of patients received ADR as first-line chemotherapy (74%) and combined with other anticancer drugs (91.4%). The mean total ADR dose was 268 mg/m^2 (range, 15–1251 mg/m^2), and 64 patients received > 550 mg/m^2 (5.1%). The difference between the total number of evaluable patients and the reported number is due to the lack of some minor information. The number of patients who received a total ADR dose of > 550 mg/m^2 is reported for each subgroup. At the time of this analysis, 716 patients had died, while 236 were alive and 314 were lost to followup.

Adriamycin-Related Cardiomyopathy

The total incidence of "definite" ADR-induced CMP was 1.7% and all of these 22 patients were affected by congestive heart failure (CHF). In addition, 38 patients (3.0%) were reported as having "possible" ADR-induced CMP. This group included 22 patients with CHF and 16 with other cardiac complications such as myocardial infarction, cardiogenic shock, cardiac arrest, increased heart size, and symptomatic cardiac arrhythmia.

The characteristics of CMP are detailed in table 2. "Definite" ADR-CMP caused death in eight of 1266 evaluable patients (0.6%) while "possible" ADR-CMP caused death in 13 patients (1.0%). CMP was clinically detected in more than one half of the patients after the ADR treatment had been discontinued. The median time which elapsed from the last dose of ADR to the first signs of CMP was 1 month for both groups, and non-significant differences were observed between survivors and nonsurvivors.

Survival time was taken into consideration only in patients who died from CMP and was calculated from when the first sign of cardiac failure was observed. Six of 22 patients died from a "definite" ADR-CMP with a median survival time of 32.5 days (range, 3–1053 days), while ten of 38 patients died from a "possible" ADR-CMP with a median survival time of 3.5 days (range, 0–26 days). In addition, the CMP data were analyzed with other available information in an attempt to detect any possible correlation.

Analysis of Other Variables

(*a*) *ADR total dose.*—The incidence of both "definite" and "possible" ADR-CMP expressed as a function of ADR total dose is shown in figure 2. Both series of data were analyzed after logit transformation (4). For "definite" ADR-CMP a highly significant curvilinear regression was obtained, while "possible" ADR-CMP was not found to be dose-related and is represented by a line corresponding to the overall incidence in this group (3%). For "definite" ADR-CMP the regression on the dose was found to be equally evident when the incidence for each dose level was calculated for all of the patients who received that dose (including those patients who continued treatment and thus reached a higher total dose). In any case, it is apparent that the increase in the slope of the toxicity curve appears in the dose range of 450–550 mg/m^2.

The following comments will concern only "definite" ADR-CMP, although the results related to "possible"

TABLE 2.—Characteristics of CMP

	"Definite" ADR-CMP	"Possible" ADR-CMP
Occurrence (No. of patients)		
During ADR treatment	8	15
After ADR treatment	14	20
Unknown	0	3
Course (No. of patients)		
Totally resolved	1	5
Resolved residually	6	6
Stable but unresolved	6	9
Death from CMP	8	13
Unknown	1	5
Median time in days (range) from last dose of ADR to CMP		
All patients with CMP	30 (0–309)	29 (1–1307)
Patients who died	29 (0–80)	37 (2–990)
Patients who survived	38.5 (0–309)	29 (1–1307)

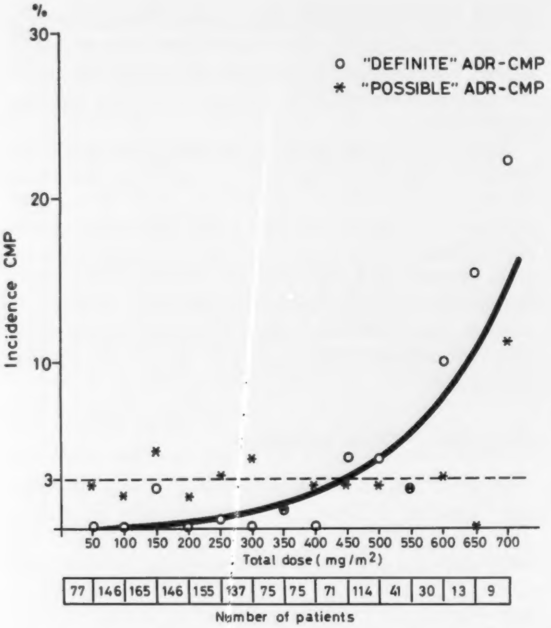

FIGURE 2.—Effect of total dose on incidence of CMP in all patients receiving ADR.

ADR-CMP are shown in tables 3–5. A comparative analysis of possible co-factors of both "definite" and "possible" ADR-CMP is shown in table 6.

(*b*) *ADR schedule.*—The data related to the ADR schedule (table 3) showed a lower incidence of "definite" CMP in the groups of patients who received ADR as a single dose every 4 weeks and on a weekly regimen. In both groups low total ADR doses were given. Statistical analysis failed to show a significant difference in the incidence of "definite" CMP among the various schedules considered.

(*c*) *Type of administration.*—The drug was given as a bolus iv injection in 1074 patients and as an iv infusion (> 30 minutes' duration) in 182 patients. The incidence of "definite" ADR-CMP was 2% in the former group and 0 in the latter. The difference is close to significance ($P = 0.1$). However, it should be noted that 58 of 1074 patients and only four of 182 patients received a total dose of ADR > 550 mg/m^2.

(*d*) *Other cytotoxic treatments.*—After having grouped together the patients who received each cytotoxic treatment, whether before or concomitantly with ADR, the following drugs were found to be of interest: cyclophosphamide (CYC), vincristine (VCR), bleomycin (BLM), and cytosine arabinoside (Ara-C). Data related to RTm were also taken into consideration (table 4). The chi-square test was carried out between pairs of subgroups, less than and greater than a total ADR dose of 550 mg/m^2. The incidence of CMP in each subgroup was compared with that of the remaining patients and a significant difference was found in the following subgroups: (*a*) when VCR was given both before and concomitantly with ADR; (*b*) when BLM was given before ADR; and (*c*) when RTm was given concomitantly with ADR.

(*e*) *Sex, age, and type of malignancy.*—The incidence of "definite" CMP was higher in females than in males although the difference is not statistically significant. After a comparison of the subgroups by age and total dose of ADR, no statistical difference was found. The incidence of "definite" CMP was 3.3% in patients who were > 60 years old and 1.3% in younger patients. In the subgroups of patients receiving > 550 mg/m^2 the incidence of CMP was 13.3% in patients who were > 60 years old and 8.6% in younger patients (table 5). Among patients with hematologic malignancies, the highest incidence of "definite" ADR-CMP was observed in the subgroup with acute myeloblastic leukemia (3.5%), while among patients with solid tumors, the incidence was highest in the lung cancer group (4%).

(*f*) *Baseline liver function tests, blood pressure, and ECG.*—Baseline liver function tests were considered im-

TABLE 3.—CMP: possible treatment co-factors

	Total No. of patients	No. of patients (%) who received ⩾ 550 mg/m^2 of ADR	No. of patients (%) with— "Definite" ADR-CMP	No. of patients (%) with— "Possible" ADR-CMP
ADR schedule				
Single dose every 3 wks	594	40 (6.7)	15 (2.5)	24 (4.0)
Single dose every 4 wks	81	1 (1.2)	1 (1.2)	2 (2.5)
Daily dose × 3 every 3 wks	41	2 (4.9)	1 (2.4)	2 (4.9)
Weekly	69	0 (0)	1 (1.4)	2 (2.9)
Other	259	20 (7.7)	2 (0.8)	4 (1.5)
Route of administration				
Bolus iv	1074	58 (5.4)	22 (2.0)	34 (3.2)
Infusion iv	182	4 (2.2)	0 (0)	2 (1.1)

paired when SGOT was more than twice the normal value, and/or the bilirubin was > 1 mg/100 ml, and/or the BSP was > 9%. Other laboratory tests (LDH, prothrombin time, and serum proteins) were available only in a few cases and therefore the results were not taken into consideration. The incidence of "definite" ADR-CMP was 1.7% when liver function was normal and 1.9% when biochemical tests were abnormal. No difference in the incidence of "definite" ADR-CMP was found between the patients with a blood pressure of ≤ 140/90 (1.8%) and those

TABLE 4.—CMP: possible chemotherapeutic co-factors

	No. of patients with CMP/No. with or without additional therapy			
	"Definite" ADR-CMP		"Possible" ADR-CMP	
	< 550 mg/m²	≥ 550 mg/m²	< 550 mg/m²	≥ 550 mg/m²
No CYC	11/653	4/34	23/653	0/34
CYC*	4/562	2/27	13/562	2/27
No VCR	2/345	3/26	11/345	1/26
VCR*	13/855	4/38	25/855	1/38
VCR: only both before and concomitantly with ADR	4/83†	—	2/83	—
No BLM	12/1011	6/51	32/1011	1/51
BLM*	3/189	1/13	4/189	1/13
BLM: only before ADR	3/48†	0/2	0/48	0/2
No Ara-C	13/1161	6/61	35/1161	2/61
Ara-C*	2/39	1/3	1/39	0/3
No RTm	8/983	4/44	27/983	1/44
RTm*	7/217†	3/20	9/217	1/20
RTm: only concomitantly with ADR	3/50†	2/11	3/50	0/11

*Includes patients who received therapy either before, concomitantly with, or before and concomitantly with ADR.

†Significant difference was observed (*see* text).

TABLE 5.—CMP: possible patient characteristic co-factors

	Total No. of patients	No. of patients (%) with—	
		"Definite" ADR-CMP	"Possible" ADR-CMP
Sex			
Males	574	6 (1.0)	15 (2.6)
Females	692	16 (2.3)	23 (3.3)
Age in yrs			
< 15	110	1 (0.9)	0 (0)
15–29	150	0 (0)	1 (0.7)
30–44	239	4 (1.7)	4 (1.7)
45–59	421	7 (1.7)	18 (4.3)
≥ 60	338	10 (3.0)	15 (4.4)
Type of cancer			
Hematologic	322	5 (1.6)	8 (2.5)
Solid tumor	933	17 (1.8)	30 (3.2)
Liver function*			
Normal	881	15 (1.7)	22 (2.5)
Impaired	208	4 (1.9)	8 (3.8)
Blood pressure*			
≤ 140/90	1119	20 (1.8)	26 (2.3)
> 140/90	121	2 (1.6)	10 (8.3)
ECG*			
Normal	732	13 (1.8)	20 (2.7)
Impaired	247	8 (3.2)	14 (5.7)

*Baseline values.

TABLE 6.—Analysis of possible co-factors related to patients, disease, and treatment: significance of correlations with ADR-CMP

	Significance with—	
	"Definite" ADR-CMP	"Possible" ADR-CMP
Sex	NS*	NS
Age	NS	NS
Cancer type	NS	NS
Baseline liver function	NS	NS
Baseline hypertension	NS	$P < 0.01$
Baseline ECG alterations	NS	$P < 0.05$
Total dose	$P < 0.01$	NS
ADR schedule	NS	NS
Type of administration	$P = 0.10$	NS
VCR (only before and concomitantly with ADR)	$P < 0.01$	NS
BLM (only before ADR)	$P < 0.05$	NS
RTm (overall)	$P < 0.05$	NS
RTm (only concomitantly with ADR)	$P < 0.05$	NS

*NS = not significant.

with a blood pressure of > 140/90 (1.6%). The baseline ECG was impaired in 247 patients and normal in 732. "Definite" ADR-CMP was observed in 3.2% and 1.8%, respectively. The differences are not significant (table 5).

(*g*) *ECG changes.*—All ECG changes observed during the period of ADR treatment were recorded. Clearly, the frequency of these abnormalities was higher when the ECG was recorded more often. The incidence fell from 29.2% (when the ECG was monitored at each ADR course) to 14% (when the ECG was repeated only before ADR and at the end of ADR treatment) (table 7). With the aim of obtaining a homogeneous group, patients in whom the ECG was repeated after every one or two courses were analyzed separately (table 8). The overall incidence of ECG changes in this group was 29.0%. Abnormalities consisted mainly of nonspecific changes of the ST tract and of the T wave, sinus tachycardia, and arrhythmias. The observed alterations were completely resolved in 46 patients (45.1%), disappeared and later reappeared in 11 (10.8%), remained stable in 29 (28.4%), and progressed in ten (9.8%). In 14 patients the outcome was unknown. Eight of 379 patients of this selected group developed "definite" ADR-CMP: three of 269 in whom no ECG changes were recorded and five of 110 who showed some ECG alterations. This difference according to the chi-square test is not significant.

DISCUSSION

When the present study was planned, only data relative to some aspects of CMP related to ADR monochemotherapy were known and only a few indications were available in relation to combination chemotherapy. Moreover, there was not, nor is there today, any noninvasive method which proved to be definitely predictive for ADR-CMP (5). In fact, endomyocardial biopsy (6) is still not easily accepted by all patients or feasible by the majority of centers that treat cancer with ADR. Although not entirely conclusive, the present retrospective study provides some new data and suggests some hypotheses to be tested in future prospective clinical trials. The advantage of this study lies in the fact that it involved a large amount of data collected from oncologic institutions where polychemotherapy is routinely applied by trained oncologists and where ADR is one of the standard drugs. By taking advantage of the specific experience of the physicians involved in this study it was possible to further subclassify the CMP as "definite" or "possible." In fact, by analyzing separately the two types of CMP, it was possible to identify factors which are more clearly associated with the actual drug-related CMP.

TABLE 7.—ECG changes during ADR treatment

ECG repeated—	Total No. of patients	No. of patients (%) with ECG changes
At each course	267	78 (29.2)
Every 2 courses	112	32 (28.6)
Every 3–4 courses	40	5 (12.5)
After end of ADR	93	3 (14.0)
Never	65	—
Unknown	689	—

A limitation of this study, on the other hand, is that the influence of each variable was assessed individually, and no formal analysis of possible interactions was carried out. The total incidence of ADR-related CMP (1.7%) appears to be in the same range as that presently known for ADR monochemotherapy. The reasons why the role played by ADR was considered to be doubtful or ancillary were specified by the physicians in most cases which were reported as "possible" CMP. However, even if patients classified as "possible" CMP are considered as having true ADR-CMP, the total incidence is not > 4.7%. Obviously, this level of late toxicity has to be weighed against the advantage offered by the drug given in a combination treatment, since ADR is a frequent component of several useful regimens. It should also be remembered that the CMP itself caused death only in about one third of the cases, while in > 50% of the CMP cases the registered cause of death was disease progression. Data on the histopathology of the heart were insufficient

TABLE 8.—Type of changes in patients with ECG repeated every 1 or 2 courses

ECG changes	No. of patients
ST-T wave changes (nonspecific)	33
Sinus tachycardia	27
Low voltage of T wave	24
Atrial tachyarrhythmia	15
Abnormal T axis	13
Premature ventricular contractions	11
Low voltage of QRS complex	9
Right bundle branch block	3
Left or right atrium enlargement	3
Left axis deviation	2
Right axis deviation	2
Ectopic atrial beats	2
Ventricular tachyarrhythmia	2
1st-degree block	2
Left bundle branch block	1
Abnormal T wave	1
T-wave inversion	1
Premature atrial contractions	1
Other	7

to draw any conclusion on possible relationships between clinical and histopathologic diagnosis of ADR-CMP.

This study confirms the value of the empiric clinical decision to keep the total cumulative dose in the range of 550 mg/m^2 as a threshold for a fully acceptable risk. However, as could be reasonably expected on theoretic grounds, the analysis of the total dose probability curve indicates, as far as "definite" CMP is concerned, that there is no absolute cut-off point for the total dose of ADR which should not be exceeded under any circumstance. This appears to be supported also by the reported patients who received total doses of $\geq$ 1–2 g/m^2 of ADR without developing CMP (2,3). Rather, there is a continuous increase in the risk with the increasing total dose of ADR. On the contrary, with "possible" CMP, the probability of developing CMP appears to remain the same at each dose level. In our opinion, these results indicate that other variables may play an important role in the onset of the CMP. Correlation studies carried out in our series of data led to a possible definition of some characteristics within the treated population and related to patient management which may be identified as "co-factors." It is noteworthy that co-factors related to preclinical conditions (predisposing co-factors), such as hypertension or baseline ECG alterations, were significantly associated with "possible" ADR-CMP, while some co-factors related to treatment (iatrogenic co-factors) were significantly associated only with "definite" ADR-CMP. Obviously, this distinction between predisposing and iatrogenic co-factors must be kept in mind when planning new, prospective controlled clinical studies. While predisposing co-factors should be stratification parameters, the possible influence of the suggested iatrogenic co-factors should become the specific objective of studies designed to minimize the risk of ADR-induced CMP. Besides RTm, many cytotoxic drugs have been reported to be cardiotoxic to some extent in man (7–13). Contrary to what is often believed (14), CYC failed to produce a synergistic myocardiotoxic effect with ADR in our study. It was however, confirmed that concomitant radiotherapy to the mediastinal-pericardial area increased the risk for ADR total doses of $<$ 550 mg/m^2. With VCR, the significantly higher incidence of CMP only in the group who received the drug both before and concomitantly with ADR points out the possible cardiotoxic role of either a long-term and/or a high cumulative-dose treatment with VCR. The preliminary observations on the role of VCR, as well as BLM and Ara-C, need to be confirmed in a larger series of patients. Recent studies reported in the literature have shown a reduced incidence of delayed cardiotoxicity when ADR is given by a weekly schedule instead of by the most widely used 3-week regimen; this has been confirmed also by the studies of Weiss et al (15) and Von Hoff et al (16). A reduced incidence of CMP for the weekly regimen was also observed in the present study, but the difference was not significant, probably because the number of patients in this group was too small compared to the number of patients who received a single dose every 3 weeks. Moreover, a significantly lower ADR total dose was given in the weekly regimen group. That no cases of CMP were seen when ADR was given as an iv infusion is rather impressive and deserves further consideration. Following bolus administration, ADR reaches a peak level in the blood which is proportional to the injected dose; the initial blood levels then decline rapidly, suggesting rapid uptake by tissues (17). No pharmacokinetic data are available for ADR administration at different infusion rates. However, it is easily understandable that, at equal dose levels, the longer the time of infusion, the lower the peak level. Since blood levels should correlate with cardiac uptake, which appears to be a major factor for further development of CMP, the above data confirm the need for a deeper pharmacokinetic approach to the problem of cardiotoxicity of the anthracyclines. In a recent study carried out in the Farmitalia Research Laboratories (Bertazzoli C, personal communication) to evaluate ADR chronic toxicity in rats treated by either iv bolus injection or iv slow infusion, it has been shown that slow administration reduces the cumulative toxicity, especially at the level of the heart and kidney. The possibility of reducing the risk of cardiotoxicity by modifying the schedule or the type of administration could represent a new way of overcoming this important side effect, providing the antitumor activity remains unchanged. This study does not provide any conclusive data on the possible predictive value of ECG changes. In our opinion, no indication will be provided even if a larger number of patients are considered, since the incidence of observed ECG changes is clearly related to the frequency of ECG monitoring; only prospective monitoring studies may elucidate this.

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Experimental Evaluation of Anthracycline Analogs

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SUMMARY

This review summarizes the preclinical tests conducted to date for experimental evaluation of new anthracycline analogs. Most of the data are derived from the experience at the Istituto Nazionale Tumori, Milan, Italy, at the National Cancer Institute, Bethesda, Md, and at the Farmitalia Research Laboratories, Nerviano, Italy. In vitro cytotoxicity tests are useful for determining the doses to be used in vivo. Antitumor activity tests in mice can be divided into different stages. P388 and L1210 leukemias are generally used in primary screening; the value of adding L1210 leukemia is briefly discussed. Other experimental tumors adopted include disseminated leukemia and transplanted solid tumors. The importance of the route and schedule of treatment is stressed. Drugs should be administered iv in the case of solid tumors, and the schedule of treatment can be adjusted according to the pharmacokinetic properties of the new analog, when these are known. If possible, the parent compound and the new analog should be dissolved in the same solvents. In the toxicity tests, cardiac toxicity deserves particular attention. Until now, the only experimental model in which a number of new anthracyclines have been tested is the rat model proposed by Zbinden. A comparison between cardiotoxicity data obtained in such models and antitumor data obtained in mice shows that cardiac toxicity can be dissociated from the antitumor activity. Knowledge of pharmacokinetic properties of new analogs is of importance for selecting the schedules of treatment and for explaining selective toxic effects.

[Cancer Treat Rep 63:835–844, 1979]

Evaluation of the biologic properties of new anthracyclines poses several questions that differentiate this type of study from those on new antitumor agents. It is necessary to compare new analogs with the two anthracyclines already successfully used in the clinic, daunorubicin and Adriamycin. Experimental studies should be directed at finding compounds that are active in resistant tumors or show a higher therapeutic index in responsive tumors.

To seek these final goals, several experimental methods have been adopted in various laboratories and it would be difficult to summarize all of them here. This paper describes the experience gained during the last 10 years in the study of a large number of anthracycline derivatives synthesized at the Farmitalia Research Laboratories (Milan, Italy) and evaluated at the Istituto Nazionale Tumori (Milan, Italy) and at the Farmitalia Research Laboratories (Nerviano, Italy). These methods will be compared to those used in other laboratories.

INTRODUCTION OF THE COMPOUND

Figure 1 summarizes the tests to which a new analog is submitted. The primary tests concern the cytotoxic activity and the antitumor activity in mice. On the basis of the information gained here, a number of additional tests are developed. The panel of tests includes toxicologic, pharmacologic, and biochemical studies developed in various laboratories.

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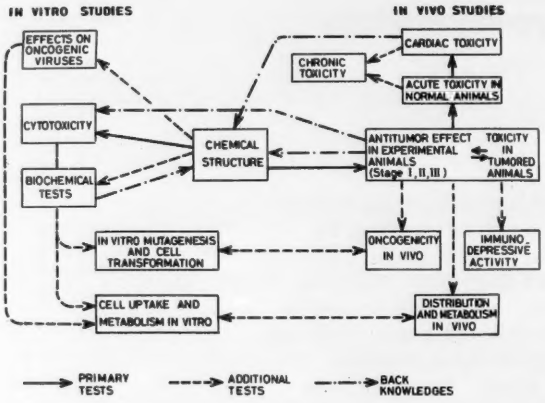
IN VITRO STUDIES
IN VIVO STUDIES
EFFECTS ON ONCOGENIC VIRUSES
CYTOTOXICITY
BIOCHEMICAL TESTS
CHEMICAL STRUCTURE
CARDIAC TOXICITY
CHRONIC TOXICITY
ACUTE TOXICITY IN NORMAL ANIMALS
ANTITUMOR EFFECT IN EXPERIMENTAL ANIMALS (Stage I, II, III)
TOXICITY IN TUMORED ANIMALS
IN VITRO MUTAGENESIS AND CELL TRANSFORMATION
ONCOGENICITY IN VIVO
IMMUNO DEPRESSIVE ACTIVITY
CELL UPTAKE AND METABOLISM IN VITRO
DISTRIBUTION AND METABOLISM IN VIVO
PRIMARY TESTS
ADDITIONAL TESTS
BACK KNOWLEDGES

FIGURE 1.—Preclinical studies on anthracycline analogs at the Istituto Nazionale Tumori, Milan, Italy, and at the Farmitalia Research Laboratories, Nerviano, Italy.

TABLE 1.—Protocol for testing new anthracycline derivatives in vitro at the Instituto Nazionale Tumori, Milan, Italy

Stage	Test system	Criterion for activity
I	HeLa cells	Colony inhibition
II	MEF* stimulated by FCS†	Inhibition of ^{3}H-TdR incorporation into DNA
III	MEF ± MSV-M‡	Inhibition of foci formation
	MS-2, MS-2T	Inhibition of colony formation and MI§
	3T3, 3T12, SVT2	Inhibition of colony formation and MI§

*Mouse embryo fibroblasts (secondary cultures).
†Fetal calf serum.
‡Murine sarcoma virus-Moloney.
§Mitotic index.

BIOCHEMICAL TESTS

The major biochemical tests in cell-free systems for the study of new anthracycline analogs and the comparison of the results obtained in biochemical and biologic tests have been previously discussed (1,2). As expected, the conclusion of this study was that DNA-binding capacity and interaction specificity are probably necessary but not sufficient conditions for antitumor activity, and that a study of the pharmacologic properties of these molecules would be critical to establishing a basis of antitumor activity and selectivity.

CYTOTOXICITY TESTS

The tests adopted in our laboratories are summarized in table 1. The colony inhibition test on HeLa cells (24-hour treatment) remains the primary evaluation model in vitro since it gives consistent and reproducible data. The results correlate well with the potency of the compounds in mice and can therefore be used to determine in vivo doses. A comparison between the cytotoxicity on mouse embryo fibroblasts and the in vivo potency showed a good correlation in the case of a series of 4′-epi derivatives of daunorubicin and Adriamycin (1). A less strict correlation was found in a series of 4-demethoxy derivatives: 4-demethoxydaunorubicin was 66 times more active than daunorubicin against HeLa cells in vitro, but it was only four times more potent than the parent compound in L1210 leukemia-bearing mice[3] (table 2). On the contrary, 1,4-dichloro-4-demethoxydaunorubicin was about as active as daunorubicin in vitro, but it did not show any antitumor activity in mice even at the maximal tolerated dose. This suggests that the compound can be inactivated in vivo.

The other tests described in table 1 are designed to give information on the mechanisms of the inhibition of cell proliferation. With regard to the inhibition of thymidine incorporation, no strict correlation was found in the case of 4-demethoxy derivatives between cytotoxicity and inhibition of nucleic acid synthesis (3). Such a correlation was found, however, in the case of a series of 4′-epi derivatives (1,4). This suggests that additional tests should be employed in order to better explain the mechanism of action of these compounds. Two sets of cultures (MS-2, MS-2T; and 3T3, 3T12, SVT2) have been introduced in order to ascertain in vitro whether the new analogs have different activities against cells with different oncogenicity. Both daunorubicin and Adriamycin inhibit the formation of foci of transformed cells induced by the murine sarcoma virus-Moloney (MSV-M) (5), but none of the analogs tested clearly showed selective antiviral activity; therefore, this test is not carried out routinely.

ANTITUMOR ACTIVITY TESTS

Experimental Tumors

The methods adopted at the Istituto Nazionale Tumori, Milan, Italy, for the evaluation of antitumor activity in mice are reported in table 3. It is known that daunorubicin and Adriamycin are more active against P388 leukemia than against L1210 leukemia (6). Treatment against early P388 leukemia results in a high number of long-term survivors; therefore, with Adriamycin derivatives, it is sometimes rather difficult to detect small differences. In L1210 leukemia, the increase in lifespan (ILS) is smaller and more reproducible than in P388 leukemia,

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[3] Di Marco A, Casazza AM, Soranzo C, et al. Effect of various substitutions in positions 1, 2, 3, and 4 of 4-demethoxydaunorubicin and 4-demethoxyadriamycin. Cancer Chemother Pharmacol. In press.

TABLE 2.—Comparison between in vitro and in vivo activity of 4-demethoxy derivatives

	Cytotoxic activity		Antitumor activity		
Compound	Concentration (ng/ml) required for 50% inhibition of HeLa cells*	R†	Dose‡	R†	T/C§ (%)
Daunorubicin	10.00		4		150
4-Demethoxydaunorubicin	0.15	66	1	4	150
2,3-Dimethyl-4-demethoxydaunorubicin	5.80	1.7	1.25	3.2	131
(2,3-*b*)-Benzo-4-demethoxydaunorubicin	27.00	0.37	10	0.4	135
2,3-Dichloro-4-demethoxydaunorubicin	25.00	0.40	33	0.12	111
1,4-Dimethyl-4-demethoxydaunorubicin	10.05	1	6.6¶	0.6	147
1,4-Dichloro-4-demethoxydaunorubicin	7.15	1.39	20	0.2	116
Adriamycin	15.00		2.9		141
4-Demethoxyadriamycin	0.10	150	0.5	5.8	166
2,3-Dimethyl-4-demethoxyadriamycin	7.00	2.14	4.4	0.6	173

*HeLa cells were exposed to the drugs for 24 hrs.

†Ratio between dose of daunorubicin or Adriamycin and dose of derivative.

‡Optimal (non-toxic) dose (mg/kg) in mice bearing L1210 leukemia treated ip on Day 1.

§Median survival time of treated mice/median survival time of control mice, × 100.

¶Maximal dose tested.

TABLE 3.—Protocol for testing new anthracycline derivatives in experimental murine tumors at the Istituto Nazionale Tumori, Milan, Italy

	Test system			Drug treatment		
Stage	Mouse strain	Tumor	Route	Route	Schedule	Criteria of activity*
I	BDF_1	L1210 leukemia	ip	ip	Day 1	ILS, LTS
	BDF_1	P388 leukemia	ip	ip	Day 1	ILS, LTS
II	C3H/He	Gross leukemia	iv	iv	Days 1, 2, 3	ILS
				Oral	Days 1, 2, 3	ILS
	CD-1	Solid sarcoma 180	sc	iv	Days 1–5	Tumor growth, ILS
III	C3H/He	Mammary carcinoma (3rd generation)	sc	iv	Different for early and advanced tumor	Tumor growth, ILS
	BALB/c	MS–2	im	iv	Different for early tumor, for advanced tumor, and after surgical removal	ILS, tumor growth, lung metastases
	CDF_1	Lewis lung carcinoma	im	iv	Different for early tumor, for advanced tumor, and after surgical removal	ILS, tumor growth, lung metastases
	CD-1	MSV-M-induced sarcoma	im	iv	Days –3, –2, –1	Tumor regression
	BALB/c				Days 3–10	Tumor growth
	BALB/c	MLV leukemia	iv	iv	Different for early and advanced tumor	ILS, No. of tumor cells (bioassay)
	BALB/c	MSV-PV infection	ip	iv	Different for early and advanced tumor	Splenomegaly, viremia

*ILS = increase of median survival over untreated controls; LTS = long-term survivors.

TABLE 4.—Effect of 4′-*O*-methyladriamycin and Adriamycin against L1210 and P388 leukemias in mice

Compound	Dose* (mg/kg)	L1210†,‡			P388‡		
		ILS	LTS	Toxicity	ILS	LTS	Toxicity
Adriamycin	4.4	69	1/10		154		
	6.6	69 (58–75)	5/28		141	3/10	
	10.0	84 (79–87)		3/28	120	5/10	
4′-*O*-Methyladriamycin	2.9	74			160	2/10	
	4.4	168 (105–212)	7/28	1/28	100	2/10	
	6.6	135 (100–175)	7/28	3/28	270	3/10	3/10
	10.0	–31 (–25, –38)		17/18			

*Treatment given ip on Day 1.

†Data from 3 experiments.

‡ILS = % increase in median survival time (dead mice only); LTS = long-term survivors (> 60 days)/No. of treated mice; toxicity = No. of toxic deaths/No. of treated mice.

TABLE 5.—Activity of daunorubicin and Adriamycin against MS-2 tumor

Drug	Days of treatment	Dose (mg/kg/day)	% inhibition of tumor growth*	ILS (%)
Daunorubicin	1, 4, 7, 10, 13	6	61†	29‡
	1, 8, 15, 22, 29	5.3	40	24†
		8	39	15
		12	65†	–47
Adriamycin	1, 4, 7, 10, 13	4	69‡	31‡
	1, 8, 15, 22, 29	10	76‡	1
	6, 10, 14, 18	2.5	42	19
		3.5	68†	32‡
	6, 10, 14, 18	3.25	8	8
		4.0	38	7

*Determined at 25–33 days.

†$P < 0.05$ (Student's t test) as compared to untreated controls.

‡$P < 0.01$ (Student's t test) as compared to untreated controls.

there are only occasional long-term survivors, and small differences can be more easily detected. L1210 leukemia, even if not recommended for screening new antitumor drugs, can be a useful model for the study of anthracyclines. We have recently identified a new Adriamycin derivative, 4′-*O*-methyladriamycin,[4,5] that is significantly more active than the parent compound against L1210 leukemia but not against P388 leukemia (table 4).

Stage II includes tests against disseminated Gross leukemia and solid sarcoma 180. Treatment is carried out by the iv route or orally.

Stage III includes transplanted solid tumors and virus-induced tumors. As a model for mammary carcinoma, the third-generation transplant of spontaneous mammary carcinoma in C3H mice has been adopted (7). In this system Adriamycin has remarkable antitumor activity, producing inhibition of tumor development and regression of already established tumors of up to 90%. The activity depends on the schedule of the iv treatment, and the most effective treatment is every other day × 12 with a low dose (2–2.5 mg/kg) (7,8) or treatment on a weekly schedule for 3–4 weeks with a high dose (6 mg/kg) (9).

The origin and characterization of the MS-2 sarcoma and the advantages of its use have been reported previously (10). Adriamycin has schedule-dependent activity on both the transplanted tumor growth (table 5) and the lung metastases (11). This experimental model has been applied to the study of some analogs. 4′-Epiadriamycin gave a higher number of complete cures (evaluated by bioassay of the lungs) than did Adriamycin (9).

The MSV-M-induced tumor has been used in our laboratory for the differentiation of the antitumor and im-

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[4] Cassinelli G, Ruggieri D, and Arcamone F. Synthesis and antitumor activity of 4′-*O*-methyldaunorubicin, 4′-*O*-methyladriamycin and their 4′-epi-analogues. J Med Chem. In press.

[5] Johnson RK, Goldin A, Di Marco A, et al. Activity against ascitic leukemias of Adriamycin derivatives modified in the 4′ position of the aminosugar moiety. Manuscript in preparation.

munodepressive effects of daunorubicin and Adriamycin and of their derivatives (8,12). The last two models mentioned in table 2 have not been used as extensively as the others and are only occasionally employed.

The tumor models adopted at the National Cancer Institute (Bethesda, Md) for testing new anthracycline analogs include P388 and L1210 leukemias, B16 melanocarcinoma, Lewis lung carcinoma, $CD8F_1$ and 16/C mammary adenocarcinomas, colon adenocarcinomas 26 and 38, and human xenografts in nude mice. Again, the primary screening is carried out on ascitic leukemias, whereas a better characterization of the active compounds is obtained by tests against solid tumors. Other experimental tumors have been utilized by different research groups but their approaches have been generally very similar.

What becomes evident from this review is that tumor lines that have acquired resistance to Adriamycin and daunorubicin are not routinely used. The fact that some analogs have shown higher activity than Adriamycin on resistant lines (11,13) and the clinical relevance of an eventual activity suggest that tests against resistant lines should be carried out more intensively.

Route of Administration

Anthracyclines are less toxic and more active against solid tumors when administered iv than ip (14). Furthermore, the iv route is the one utilized in clinical practice. For these reasons, iv administration should be employed as often as possible for treatment of solid tumors and disseminated leukemias, whereas in the case of ascitic leukemias ip administration is the most appropriate.

Adriamycin is not active when given orally (15). Clinically, ulcers produced by extravasation of the drug outside the vein of injection, even if they usually result from improper administration, can be a significant complication. The use of analogs active by the oral route can help to avoid this toxic effect and can result in a different spectrum of toxicity and antitumor activity, due to the different pharmacokinetics that result after oral and iv treatment.

Until now only a few anthracyclines have been reported to be active orally: carminomycin (16) and the 4-demethoxy derivatives of daunorubicin and Adriamycin (15).[6] Aclacinomycin A has been reported to be absorbed well after oral administration (17).

A comparison of the antitumor activity of some anthracyclines administered orally to Gross leukemia-bearing mice is reported in table 6. At a non-toxic dose, semisynthetic carminomycin (18) was not active, 4-demethoxydaunorubicin and 4-demethoxyadriamycin significantly increased the lifespan of mice, and daunorubicin was active but at very high doses (25–50 mg/kg/day).

TABLE 6.—Antitumor activity of anthracyclines administered orally against Gross leukemia*

Compound	Dose† (mg/kg/day)	T/C‡ (%)	No. of toxic deaths/No. of mice treated§
Daunorubicin	25	133	
	50	150	
	100	166	4/8
	200	83	8/8
Adriamycin	200	100	
4-Demethoxydaunorubicin	1.13	133	
	1.70	150	
	2.55	200	2/20
	3.82	200	11/20
4-Demethoxyadriamycin	4	117	
	5.7	133	
	8.6	183	2/15
	12.9	183	
Carminomycin	3.75	116	
	5.7	133	1/8
	8.6	216	2/8

*C3H mice were injected with 2×10^6 leukemic cells iv on Day 0.

†Treatment given orally on Days 1, 2, and 3.

‡Median survival time expressed as % of untreated controls.

§Evaluated in tumored mice on the basis of spleen and liver size reduction at necropsy.

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[6] *See* footnote 3.

TABLE 7.—Antileukemic activity of anthracyclines: effect of single treatment

Leukemia (iv)	No. of cells	Compound	Optimal dose* (mg/kg)	T/C† (%)	LTS‡
L1210	10^5	Adriamycin	12, 16	157, 162	
		4-Demethoxydaunorubicin	4.2, 4.2	343, 307	12/20
		4-Demethoxyadriamycin	2.7, 2.7	362, 371	11/20
Gross	10^2	Daunorubicin	14.4	(190)	5/6
		Adriamycin	12	190	3/6
		4-Demethoxydaunorubicin	1.7–2.5		6/6

*Treatment given iv on Day 1.
†Median survival time of treated mice/median survival time of control mice, × 100.
‡LTS = long-term survivors (> 60 days)/total No. of mice.

Schedule of Treatment

A rational approach to the choice of the schedule of treatment should be based on the pharmacologic properties of the drug and on its activity in the different phases of the cell cycle.

Adriamycin, a non-cycle phase specific agent, does not have definite schedule-dependency in ascitic leukemias. Due to the long persistence of Adriamycin in the animal body, daily treatments can result in increased toxicity. For these reasons, single or intermittent treatments (every 4 days) have been adopted. However, recent experimental and clinical results (19,20) suggest that the cardiac toxicity can be reduced by administering the drug according to different schedules of treatment. This point is of extreme relevance, and further laboratory studies are urgently needed to confirm these data.

When dealing with derivatives, the primary experimental antitumor studies are carried out before knowing the pharmacologic properties of the new drug; when these are known, the best schedule of treatment for the new analog must be tested. For example, we have studied[7] the schedule-dependence of the antileukemic activity of Adriamycin, daunorubicin, and 4-demethoxydaunorubicin, which gives higher tissue levels than does daunorubicin in most organs of mice, particularly in the spleen, and is retained longer (21). In a single-treatment schedule (Day 1), 4-demethoxydaunorubicin (as well as 4-demethoxyadriamycin) was more active than Adriamycin and daunorubicin (table 7). In repeated-treatment schedules, treatment for 3 consecutive days (Days 1, 2, and 3) was less effective for all three drugs tested than was intermittent treatment. Among the intermittent treatments, the best schedule was treatment on Days 1, 3, and 6 for daunorubicin, Days 1, 3, and 6, or 1, 4, and 7 for Adriamycin, and Days 1, 5, and 9 for 4-demethoxydaunorubicin (table 8).

Vehicles

Some anthracycline derivatives, in contrast to their parent compounds, are not water-soluble. Tween 80, at a final concentration of 10%, was extensively used in order to solubilize these drugs, and it was shown in our laboratory to potentiate the antitumor activity of Adriamycin in the iv treatment schedule but not in the ip treatment schedule, where increased toxicity was observed (22). In parallel, it has been shown that Tween 80, like amphotericin B, can partially restore the sensitivity to Adriamycin of Adriamycin-resistant tumors (23). However, Parker et al (11) did not find increased toxicity when Adriamycin was administered ip in 10% Tween 80, and Vecchi et al (24) did not find increased activity when Adriamycin was administered iv in 10% Tween 80.

These data, although contradictory, suggest that compounds altering the cell surface can modify the antitumor activity of Adriamycin, and that special care must be taken in evaluating biologic activity of anthracyclines that are not water-soluble.

RELATIONSHIPS BETWEEN ANTITUMOR ACTIVITY AND TOXICITY

General Toxicity

In mice treated with single-dose regimens the mortality caused by daunorubicin and Adriamycin is constant only after 2 or 3 months of treatment. Therefore, in antitumor activity experiments the criterion of considering toxic deaths only those that occur in the treated group before the death of the first control mouse is not suitable. In our laboratory, we carry out postmortem examinations on all dead mice, and toxicity is established on the basis of the macroscopic necroptic findings. The main findings in treated mice that die in the course of the experiment without grossly visible tumors or metastases are hemorrhagic degeneration of the intestinal mucosa and spleen and liver weight reduction.

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[7] Pratesi G, Giuliani F, et al. Unpublished data.

TABLE 8.—Antileukemic activity* and toxicity of Adriamycin, daunorubicin, and 4-demethoxydaunorubicin in normal and tumored mice: dependence on the schedule of treatment

Days of treatment	Compound	Dose (mg/kg/day iv)	T/C† (%)	LTS‡	Toxicity: Tumored mice§	Toxicity: Normal mice¶
1, 2, 3	Adriamycin	4.5	200			
		5.4	216		3/20	
		6.5	225		3/10	
	Daunorubicin	2.6	125			
		4	141			
		6	183			
		10	183		5/8	
	4-Demethoxydaunorubicin	0.5	175			
		0.75	183			
		1.13	141		4/10	
1, 3, 6	Adriamycin	4.5	566	1/8	2/8	
		5.4	658		4/8	
		6.5	250		2/8	
	Daunorubicin	6.5	166	3/8		
		7.8	675	1/8	3/8	
		9.3	433	1/8	2/8	
	4-Demethoxydaunorubicin	0.5	150		1/8	
		0.75	242	2/8	1/8	
		1.13	217		4/8	
1, 4, 7	Adriamycin	3.3	107			0/8
		5	560	2/8	1/6	0/8
	Daunorubicin	5	107			0/8
		7.5	153	2/8	1/6	0/8
	4-Demethoxydaunorubicin	0.75	215			0/8
		1.13	230		2/8	8/8
1, 5, 9	Adriamycin	3.3	121			
		5	130	2/8		
	Daunorubicin	5	114			
		7.5	130			
	4-Demethoxydaunorubicin	0.75	135			
		1.13	360	1/8		

*C3H mice were injected iv with 2 × 10^6 leukemic cells from mice bearing Gross leukemia.

†Median survival time of treated mice/median survival time of control mice, × 100.

‡LTS = long-term survivors (> 60 days)/No. of treated mice.

§No. of mice with reduction of spleen and liver size demonstrated at necropsy/total No. of mice.

¶No. of dead mice (60 days after start of treatment)/total No. of mice.

The availability of data both on antitumor activity and on toxicity allow the calculation of a therapeutic index (ratio between toxic and active doses). It was on the basis of a higher therapeutic index that Adriamycin was judged superior to daunorubicin and 4′-epiadriamycin was judged superior to Adriamycin (9).

Cardiac Toxicity

Since cardiac toxicity is an important limiting effect of the anthracyclines, a therapeutic ratio between cardiotoxic doses and antitumor doses must be evaluated. Table 9 reports such a comparison for a number of anthracycline derivatives. Since the cardiac toxicity was evaluated in the rat model (25) and the antitumor activity was evaluated in mice, to reduce the species difference the cardiotoxic and antitumor doses have been expressed in mg/m^2, according to the methods of Freireich et al (26).

These data can be criticized, since the model for cardiotoxicity evaluation and the one for antitumor activity evaluation differed with respect to animal species and routes and schedules of treatment; further studies in rabbits and mice are necessary before drawing definite conclusions. However, the ratios presented in table 9 show

TABLE 9.—Therapeutic ratio between cardiotoxic and antitumor doses of new anthracyclines

		Minimal cumulative cardiotoxic dose in rats, ip*		Gross leukemia (3 iv injections)			L1210 leukemia (1 ip injection)		
				Optimal cumulative dose			Optimal dose		
Compound	NSC No.	mg/kg	mg/kg†	mg/kg	mg/m²‡	R§	mg/kg	mg/m²	R§
AD-32	246131	48.00¶	283	90.00	288.0	0.98	~100.0	320.0	0.8
Quelamycin	267703	30.00	177	40.20	128.6	1.37	33.7	107.0	1.6
Daunorubicin	82151	13.00	76	18.00	57.6	1.33	8.0	25.6	2.9
Adriamycin	123127	11.00	64	15.00	48.0	1.35	6.6	21.1	3.0
4-Demethoxydaunorubicin	256439	1.75	10	2.25	7.2	1.43	0.6	1.9	5.4
		7.50**	44**	5.10**	16.3**	2.71**			
Adriamycin-14-octanoate	149584	20.00	118	12.30	39.3	3.00	6.2	19.8	5.9
4′-Epiadriamycin	256942	16.00	94				5.0	16.0	5.9
Adriamycin-14-*O*-stearoilglycolate	261057	40.00	236	27.00	86.4	2.73	10.0	32.0	7.3
4′-Deoxyadriamycin	267469	15.00	88	8.25	26.4	3.35	4.0	12.8	6.9
Adriamycin-14-nicotinate	149583	32.00	188	12.00	38.4	4.91			
2,3-Dimethyl-4-demethoxyadriamycin	301478	20.00	118	7.80	24.9	4.73	4.4	14.0	8.4
4-Demethoxyadriamycin	256438	3.00	17	0.90	2.8	6.14	0.5	1.6	11.0

*Data from Zbinden (personal communication and ref 25).

†Minimal cumulative cardiotoxic dose (mg/m²) = minimal cumulative cardiotoxic dose (mg/kg) × 5.9, as suggested for rats weighing 150 g (ref 26).

‡Optimal dose (mg/m²) = optimal dose (mg/kg) × 3.2, as suggested for mice weighing 24 g (ref 26).

§Ratio between minimal cumulative cardiotoxic dose and optimal antitumor dose.

¶Little evidence of cumulative cardiotoxic effect.

**Oral treatment.

that the cardiac toxicity can be dissociated from the antitumor activity (25). 4-Demethoxyadriamycin is the most potent anthracycline tested to date in our laboratory; its optimal antitumor dose is about 20 times lower than that of Adriamycin (8) and its minimal cumulative cardiotoxic dose is about 3.5 times lower than that of Adriamycin. The difference in the ratio between the respective cardiotoxic and antitumor doses results in a higher therapeutic ratio.

4′-Deoxyadriamycin, adriamycin-14-nicotinate, and 2,3-dimethyl-4-demethoxyadriamycin have optimal antitumor doses *lower* than those of Adriamycin and minimal cumulative cardiotoxic doses *higher* than those of Adriamycin. These findings might be related to significant modifications in pharmacokinetics, metabolism, or mechanism of action and warrant further investigation.

PHARMACOKINETIC PROPERTIES

The knowledge of pharmacokinetic properties, in addition to helping to understand different biologic activities, can suggest different spectrums of activity, routes of administration, and schedules of treatment. These properties can be determined by investigating cell uptake and efflux in vitro, distribution in experimental animals, excretion, and metabolism.

Cell uptake can be dependent, at least for groups of homogeneous derivatives, on the different lipid solubility, as tested by partition coefficients in *N*-butanol/aqueous buffer (2,3). However, one exception was noticed: 4-demethoxy-7,9-diepidaunorubicin (β anomer) (table 10).

Among the compounds listed in table 10, three were investigated for pharmacokinetic differences in mice: 4′-

TABLE 10.—Relationships between cell uptake in vitro and partition coefficients of anthracycline analogs

Compound	Cell uptake* (%)	Partition coefficient†
Daunorubicin	100‡	17.8
4′-Epidaunorubicin	1500	32.3
4′-Deoxydaunorubicin	300	42.0
2′-Hydroxydaunorubicin	33	11.5
4-Demethoxydaunorubicin	250	32.3
4-Demethoxydaunorubicin (β anomer)	116	11.5
4-Demethoxy-7,9-diepidaunorubicin	916	∞
4-Demethoxy-7,9-diepidaunorubicin (β anomer)	333	8.0
Adriamycin	100‡	6.3
4′-Epiadriamycin	240	32.3
4′-Deoxyadriamycin	1400	15.0
4-Demethoxyadriamycin	342	17.6

*Data from refs 2 and 3. % of cell uptake at 2 hrs after treatment in comparison with cell uptake of daunorubicin and Adriamycin.

†Partition coefficient in *N*-butanol/Tris-HCl buffer.

‡Adriamycin uptake was respectively 23% and 16% that of daunorubicin in the 2 systems employed (mouse embryo fibroblasts and L1210 leukemia cells in vitro).

TABLE 11.—Derivatives of interest identified in preclinical studies

Drugs	Activity
4′-Epiadriamycin vs Adriamycin	Equal antitumor activity, reduced toxicity and cardiotoxicity, equal potency
4-Demethoxydaunorubicin vs daunorubicin	Possibly higher antileukemic activity, activity by oral route, reduced cardiotoxic index, higher potency
4-Demethoxyadriamycin vs Adriamycin	Possibly higher antileukemic activity, equal activity in solid tumors, activity by oral route, *markedly* reduced cardiotoxic index, higher potency
4′-Deoxyadriamycin vs Adriamycin	Equal antitumor activity, reduced cardiotoxicity and cardiotoxic index, higher potency
4′-*O*-Methyladriamycin vs Adriamycin	Higher antileukemic activity (?) (L1210), slightly higher potency, reduced cardiotoxicity (mice) (?)

epiadriamycin, 4-demethoxydaunorubicin, and 4-demethoxyadriamycin. 4′-Epiadriamycin is retained less than Adriamycin in the organs (9). In solid mammary carcinoma tissue, the concentration of the two drugs was similar (9); in L1210 leukemia cells in vitro, 4′-epiadriamycin was taken up in a greater amount than was Adriamycin (2). The increased therapeutic index of 4′-epiadriamycin over Adriamycin could be explained on the basis of these findings.

4-Demethoxydaunorubicin and 4-demethoxyadriamycin showed a significantly higher partition coefficient and cell uptake than their parent compounds. In parallel, organ drug levels in mice given these 4-demethoxy derivatives were generally higher than those in mice given an equal dose of daunorubicin or Adriamycin, except in the heart (21). The higher tissue concentration in most of the organs tested, and particularly in the spleen, in comparison with the parent compounds can be correlated with the higher experimental potency in vivo, while the lack of a parallel increase at the heart level can explain the lower increase of cardiotoxicity. The higher affinity for tissues is probably the basis for the oral activity of 4-demethoxydaunorubicin and 4-demethoxyadriamycin. If rapid absorption in the gastrointestinal tract is favored, the drug will be less available to inactivation processes in the gastrointestinal fluids, particularly with regard to the split of the glycosidic bond.

These data show that pharmacokinetic studies are of importance not only for selecting the best schedule of treatment to be employed, as previously discussed, but also for an explanation, and possibly a prediction, of some selective toxic effects, such as cardiotoxicity. The metabolism of daunorubicin and Adriamycin has been extensively investigated both in experimental animals and in man. Until now only a few derivatives have been subjected to extensive metabolic investigation (27).

CONCLUSIONS

From the experience gained by the use of the experimental models described above, some anthracycline analogs showing interesting properties have been identified (table 11). In general, it was observed in our laboratory that modifications at position 4′ of the aminosugar moiety resulted in decreased toxicity (4′-epiadriamycin) and cardiotoxicity (4′-epiadriamycin and 4′-deoxyadriamycin) and higher activity against L1210 leukemia (4′-*O*-methyladriamycin). Modification at position 4 of the aglycone resulted in markedly increased potency, possibly increased antileukemic activity, activity by the oral route, and a reduced cardiotoxic index (4-demethoxydaunorubicin and 4-demethoxyadriamycin).

It is still too early to determine if these promising observations will be confirmed by clinical findings. Surely these results, as well as those obtained by others (11, 16,17,24,27), strongly indicate that modifications in the chemical structure of anthracyclines can result in relevant variations of the biologic activity, and suggest that further studies must be pursued in this promising field of antitumor chemotherapy.

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Structure-Activity Relationships Among Daunorubicin and Adriamycin Analogs

David W. Henry[1,2,*]

SUMMARY

Structural characteristics of Adriamycin and daunorubicin analogs are discussed in terms of three antitumor activity classifications: compounds with potency and efficacy equivalent to or superior to the parent antibiotics, compounds displaying high efficacy but with decreased potency, and compounds in which a key structural change has destroyed all in vivo antitumor activity. It is first concluded that analogs possessing efficacy and potency comparable to the parent antibiotics are quite similar to the parents in structural terms, ie, their hydrophilic character, functional groups, and stereochemistry. Within this group there is suggestive evidence that altered electronic character of the aglycones may be associated with improved cardiotoxicity characteristics. The second general conclusion is that high efficacy accompanied by decreased potency is compatible with a much wider array of structural variants than is high efficacy accompanied by high potency. Thus, analogs with substantially higher or lower lipophilicity than the parent antibiotics, as well as those with an array of ionic characteristics differing from the parents, retained significant efficacy. These characteristics are compatible with the concept of multiple mechanisms of action for the anthracycline antibiotics. In general, structural changes in the sugar side chain are appreciably more compatible with retention of in vivo antitumor efficacy than are changes in the aglycone, both in terms of functional groups and stereochemistry.

[Cancer Treat Rep 63:845–854, 1979]

The discovery in the 1960s that Adriamycin (ADR) *[1]* and daunorubicin (DNR) *[2]* possessed potent and useful antitumor activity has directed ever-increasing attention to their chemistry and the preparation of analogs with improved therapeutic properties. These efforts from many laboratories around the world have led to substantial progress in understanding the chemistry of these unique antibiotics. As a result, a variety of new semisynthetic anthracyclines have been advanced to clinical trial status in recent years. It is the objective of this paper to briefly review structure-activity relationships among analogs and derivatives of DNR and ADR and to attempt to interpret these results in a way that could help guide future attempts to improve on current drugs of this class.

In this report attention will be focused only on the most significant structural variations. Most of the hundreds of compounds that could be mentioned must be omitted. Only analogs directly related to ADR or DNR will be cited, although there is growing interest in a different subgroup of anthracyclines characterized by altered aglycone substitution patterns and more than one sugar in the side chain (eg, aclacinomycin A, compound *3*) (1,2) (fig 1). At present, there seems to have been very little work on synthetic modifications in this more complex group.

Discussion of antitumor test data will be restricted to those from experimental animal models. Because of the

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[2] I wish to thank Dr. Harry B. Wood, Jr, of the Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, for encouragement and helpful discussions during the course of the work. I am also very grateful to my earlier associates at SRI International, G. Tong, E. M. Acton, A. Fujiwara, W. W. Lee, D. G. Kleid, T. H. Smith, H. Y. Wu, and A. Martinez, for their permission to cite several compounds for their chemical and biologic properties (*see* footnotes to tables) prior to full publication.

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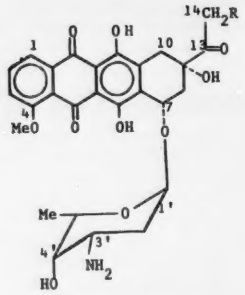
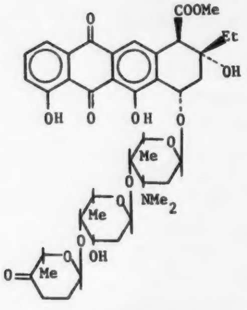


1. R = OH Adriamycin

2. R = H Daunorubicin



3. Aclacinomycin A

FIGURE 1

many different systems used for primary screening in antitumor drug development programs, it will be impossible to directly compare the activities of all compounds from various sources. To permit some degree of intercompound comparison, data for new compounds will be presented along with control data for ADR or DNR in the same test. Data from only one antitumor test system will be cited for each compound, although in many cases test results from several other systems will have been reported. Potency is reported in terms of the optimum dose (OD), the dose providing maximum increase in lifespan (ILS) for tumor-bearing treated animals. Efficacy is indicated by percent ILS, the percent ILS at the OD.

Structure-activity relationships among the anthracyclines have been reviewed previously (3–6).

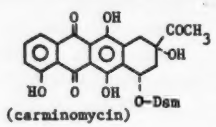
In subsequent discussion, new analogs will be classified in three ways: those with potency and efficacy equivalent to or superior to DNR or ADR, those displaying high efficacy equivalent to DNR or ADR but with decreased potency, and those in which a key structural change has destroyed all in vivo antitumor activity. Classification of the compounds into one or the other of the first two groups has been arbitrary to some extent because the potency and efficacy of a specific compound will vary from one test system to another. Potency especially can vary substantially in different systems. Nevertheless, it is believed that some useful generalizations may be gleaned from this classification which provides a convenient mechanism for condensing a large body of complex information.

Analogs With High Potency and Efficacy

Table 1 presents data on nine compounds from this group. The first compound, carminomycin ***[4]***, is a fermentation product and not a synthetic analog of DNR. However, it may be so regarded because it differs only by loss of the 4-*O*-methyl group, leaving a phenol in the 4-position. In various tests, it is about five to ten times more potent than DNR and frequently provides equal or somewhat superior efficacy. Compound ***5***, in which the 4-position is further simplified by complete deletion of the methoxy group, is also approximately five to ten times more potent than the parent (in this case, ADR) and usually of equivalent efficacy. However, the cardiotoxicity of compound ***5*** increased several-fold over that of ADR (8).

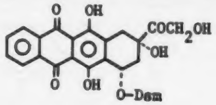
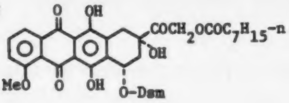
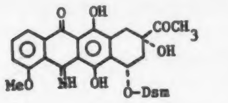
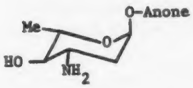
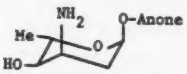
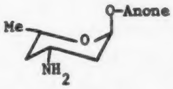
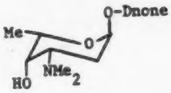
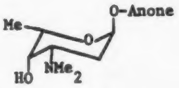
Simple esterification of the 14-hydroxyl of ADR with a variety of acyl groups results in compounds with generally equivalent potency and efficacy. In some tests, for example the mouse mammary carcinoma, some esters (eg, compound ***6***) provided results superior to ADR. The

TABLE 1.—Comparative in vivo antitumor data for ADR/DNR analogs with efficacy and potency comparable to or superior to parent antibiotics

Compound No.	Structure*	Tumor†	OD‡ (mg/kg)	% ILS§	Standard drug	OD‡ (mg/kg)	% ILS§	Ref No.
4	 (carminomycin)	L1210	2.25	> 127	DNR	15	120	7

See footnotes at end of table.

TABLE 1.—Comparative in vivo antitumor data for ADR/DNR analogs with efficacy and potency comparable to or superior to parent antibiotics—Continued

Compound No.	Structure*	Tumor†	OD‡ (mg/kg)	% ILS§	Standard drug	OD‡ (mg/kg)	% ILS§	Ref No.
5		L1210	0.5	66	ADR	5.0	66	5,8
6		Mammary carcinoma (mouse)	3.1	83	ADR	2.5	42	9
7		P388 (every day, Days 5, 9, and 13)	3.0	36	DNR	4.0	35	8¶
8		L1210	5	150	ADR	5	166	5
9		L1210	5	139	ADR	5	166	5
10		L1210	4	177	ADR	5	166	5
11		P388 (every day, Days 1–9)	2.0	114	DNR	0.78	71 ± 14	**
12		P388 (every day, Days 1–9)	0.5	62	ADR	1.0	93 ± 40	11**

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*Dsm = 1-α-daunosaminyl; Anone = 7-*O*-adriamycinonyl; Dnone = 7-*O*-daunomycinonyl.

†For details of antitumor assays, consult original references. For unpublished work from SRI International, assay data are those provided by the NCI from standard primary screens in mice.

‡OD = dose providing maximum increase in survival time.

§% ILS of treated animals over untreated controls.

¶Tong G, Henry D, and Acton EM. 5-Iminodaunorubicin, an analog with reduced cardiotoxicity. Presented at the 174th National Meeting of the American Chemical Society, Anaheim, Calif, Mar 1978 (MEDI 49).

**Henry D, Tong G, Fujiwara A, et al. Methylated analogs of daunomycin and Adriamycin. Presented at the 172nd National Meeting of the American Chemical Society, San Francisco, Calif, Aug 29–Sept 3, 1976 (MEDI 90).

active drug species, however, is very likely ADR since the ester linkage is rapidly cleaved in vivo (9). Compound *7* is 5-imino-DNR and is the result of replacing one of the carbonyl groups of the aglycone with the nitrogen-containing imino function. This compound appears to be equivalent to DNR in several primary screens, but is of more than normal interest because it elicits a substantially lower cardiotoxic response in the rat electrocardiographic (ECG) model (8).

Compounds *8* and *9* differ from ADR in the stereochemistry of the daunosamine side chain. In compound *8*, the 4′-hydroxy is equatorial, rather than axial as in ADR. In the sugar of compound *9*, both the 3′-amino and the 4′-hydroxy have been inverted from their natural configuration. Both compare quite closely with the parent in efficacy and potency. The configuration of compound *10*, another sugar-modified analog, is the same as that of ADR but the 4′-hydroxy has been deleted. Again, it is very similar to ADR.

N,N-Dimethylation of DNR provided compound *11*. This change caused efficacy to improve substantially over that of DNR, although at the expense of a small decrease in potency. The same methylation of ADR provided a compound *[12]* whose efficacy decreased appreciably but whose potency increased. However, compound *12* was found to be significantly more cardiotoxic than ADR in rats (10).

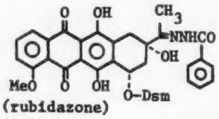
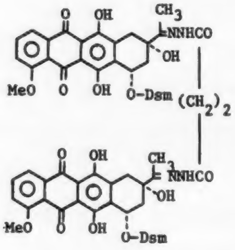
A few other compounds with efficacy and potency approaching that of the parent antibiotics might have been included in table 1, but those cited exemplify all of the types of changes compatible with optimal drug performance in these antitumor systems. Examination of the structures in table 1 reveals that none differs very much in general chemical characteristics from the parent antibiotics. All are basic and retain the skeletal structure of the parents. The lipophilic character of all of these compounds would be expected to remain approximately the same, with the exception of octanoyl ester *6*, and this derivative is known to be rapidly converted to ADR in vivo.

Analogs With High Efficacy but Reduced Potency

Table 2 lists analogs displaying antitumor effects approximately equal to, or greater than, ADR or DNR, but only at substantially higher doses. Many more compounds fall into this group than the group represented by table 1, and in many cases the example cited represents a group of analogs with roughly comparable properties. Optimal doses for this group are typically four times or more greater than those of the parent and may range up to 50 times greater.

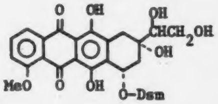
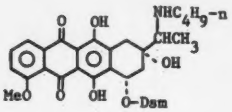
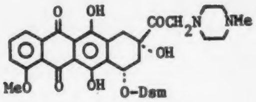
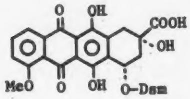
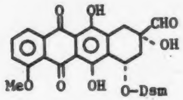
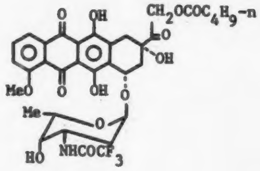
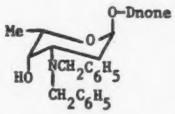
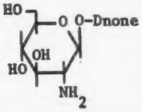
The first entry, rubidazone *[13]*, might be regarded as transitional between the high potency, high efficacy group and this group. Many hydrazones similar to rubidazone have been prepared and many show similar properties. Rubidazone is known to hydrolyze to DNR in vivo, although this appears to be a less facile transformation than the hydrolysis of ester *6*. Compound *14* is a bishydrazone designed to double intercalate into helical DNA. It is clearly more efficacious than DNR, but be-

TABLE 2.—Comparative antitumor data for ADR/DNR analogs with efficacy comparable to parent antibiotics but with decreased potency

Compound No.	Structure*	Tumor*	OD* (mg/kg)	% ILS*	Standard drug	OD* (mg/kg)	% ILS*	Ref No.
13	 (rubidazone)	P388 (every day, Days 1–9)	4	92 ± 39	DNR	0.78	71 ± 14	11,12
14	 O OH CH$_3$ NNHCO OH MeO O OH O–Dsm (CH$_2$)$_2$ O OH CH$_3$ NNHCO OH MeO O OH O–Dsm	P388 (every day, Days 1–9)	12.5	207	DNR	0.78	71 ± 14	†

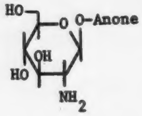
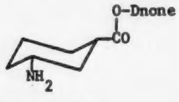
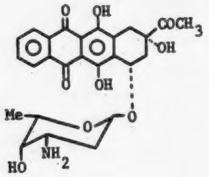
See footnotes at end of table.

TABLE 2.—Comparative antitumor data for ADR/DNR analogs with efficacy comparable to parent antibiotics but with decreased potency—Continued

Compound No.	Structure*	Tumor*	OD* (mg/kg)	% ILS*	Standard drug	OD* (mg/kg)	% ILS*	Ref No.
15		P388 (every day, Days 1–9)	8	166	ADR	1.0	93 ± 40	‡
16		P388 (every day, Days 1–9)	5	84	DNR	0.78	71 ± 14	13
17		S180 ascites	25	126	ADR	2	110	5
18		P388 (every day, Days 1–9)	25	63	DNR	0.78	71 ± 14	14§
19		P388 (every day, Days 1–9)	50	54	DNR	0.78	71 ± 14	¶
20	 (AD32)	L1210	50	> 445	ADR	4	54	15
21		P388 (every day, Days 1–9)	25	159 ± 12	DNR	0.78	71 ± 14	**
22		L1210	90	60	ADR	3.5	40	††

See footnotes at end of table.

TABLE 2.—Comparative antitumor data for ADR/DNR analogs with efficacy comparable to parent antibiotics but with decreased potency—Continued

Compound No.	Structure*	Tumor*	OD* (mg/kg)	% ILS*	Standard drug	OD* (mg/kg)	% ILS*	Ref No.
23		L1210	120	40	ADR	3.5	40	††
24	 (rubicyclamine)	L1210	10	80	DNR	0.75	80	16
25		L1210	8	50	DNR‡‡	0.5 4	50 50	5 5

*See footnotes *–§ in table 1.

†Tong G, Kleid DG, and Henry DW. Double intercalating bis-hydrazones of daunorubicin and Adriamycin. Presented at the 2nd Joint Canadian Institute of Chemistry-American Chemical Society Conference, Montreal, May 1977 (MEDI 19).

‡Test data from NCI. French patent No. 2122695 (Sept 1, 1972, to Rhone Poulenc).

§Unpublished NCI data.

¶Smith TH, Fujiwara AN, and Henry DW. Synthesis of daunomycin analogs bearing novel 9-acyl side chains. Presented at the 2nd Joint Canadian Institute of Chemistry-American Chemical Society Conference, Montreal, May 1977 (MEDI 20).

**Smith TH, Wu HY, and Henry DW. Adriamycin analogs. *N*,*N*-Dibenzyl derivatives—highly efficacious non-DNA binding antitumor anthracyclines. Presented at the 174th National Meeting of the American Chemical Society, Anaheim, Calif, Mar 1978 (MEDI 50).

††Israel M, and Murray RJ. Preparation and antitumor evaluation of β-D-glucosaminyl-daunomycinone and β-D-glucosaminyl-adriamycinone and their *N*-trifluoroacetyl derivatives. Presented at the 172nd National Meeting of the American Chemical Society, San Francisco, Calif, Aug 29–Sept 3, 1976 (MEDI 89).

‡‡This drug is 4-desmethoxy-DNR, the α-anomer of compound *25* (*see* ref 5).

cause of the ready hydrolysis of the hydrazone linkage, it is uncertain whether this superior efficacy should be attributed to different DNA-binding characteristics in vivo or simply to a repository effect caused by gradual hydrolytic release of DNR.

Reduction of the 13-carbonyl of ADR provided compound *15*. Carbonyl reduction is also known as one metabolic transformation of ADR. At eight times the dose of ADR, compound *15* provides clearly superior efficacy. Analog *16* is also a carbonyl-reduced variant, but in this case the product was formed by reductive amination so that the molecule now contains a second basic center at the 13-position. ADR derivative *17*, bearing a piperazino moiety on carbon 14, has three potentially basic centers in the molecule. Both basic modifications show efficacy at least equivalent to that of the parent.

Compound *18* (table 2), prepared by oxidative degradation of ADR, bears a carboxylic acid moiety on the 9-position, thus giving this molecule the opposite polarity to that of *16* or *17* in the region of the 9-position. Compound *18* would be expected to exist as a zwitterion in solution while *16* would be a di-cation and *17* would be a di- or tri-cation. A small neutral substituent on the 9-position, as in aldehyde *19*, is also compatible with appreciable antitumor activity.

Compound *20*, a doubly modified analog of ADR, is known as AD-32 and has recently undergone phase I clinical trials because of its outstanding efficacy in the L1210 screen. At doses about ten times greater than ADR, it effects cures in a tumor system that responds only modestly to ADR in terms of efficacy and never with cures for treated mice. AD-32 is a neutral molecule bearing an *N*-trifluoroacetyl group and a 14-*O*-valeryl ester modification. The latter group is known to be rapidly lost by hydrolytic cleavage in vivo.

Compound ***21*** is *N,N*-dibenzyl-DNR and in the P388 test system it is more efficacious than DNR or ADR at doses 25 times those of the parent antibiotics. It is estimated that compound ***21*** is $> 10^4$ more lipophilic than DNR because of the added benzyl groups. Compounds ***22*** and ***23*** are conjugates of glucosamine with daunomycinone and adriamycinone, respectively. Very large doses of these compounds are necessary but both are equivalent to or better than ADR in the L1210 system. Glucosamine is similar to daunosamine in possessing a primary amino group, but differs in the position of the amino group, in possessing three hydroxyl groups instead of one, and in several stereochemical features.

Compound ***24*** is an aminocyclohexanecarbonyl ester of daunomycinone. Aside from the differing linkage of the side chain to daunomycinone, it differs from daunosamine in stereochemistry and in lipophilicity. Several other esters bearing basic functionality have also shown substantial antitumor effects (16).

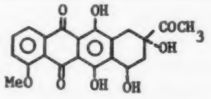
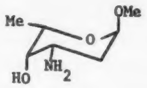
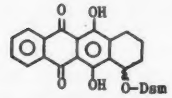
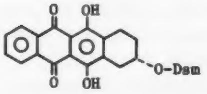
The last entry in table 2 is 1′-epi-4-desmethoxy-DNR ***[25]***. The point to be illustrated with compound ***25*** is the loss of potency upon epimerizing the 1′-position, making a β-anomer rather than the natural α-anomer. Because of the activity-enhancing effect of methoxy deletion, the effect of the anomeric change is best seen by comparing compound ***25*** with 4-desmethoxy-DNR; these data are cited in table 2 under "Standard drug." A 16-fold loss in potency with no loss in efficacy is found with this comparison.

Inactive Analogs

Compounds in table 3 have been selected to illustrate structural features whose change results in total loss of antitumor activity. Their consideration is worthwhile because they give some information on the minimum structural features necessary for activity. Data on toxicity and highest ineffective dose are provided to illustrate that the majority of inactive analogs have not lost all biologic activity, only antitumor effects.

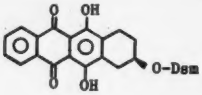
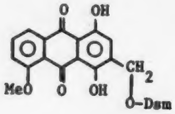
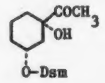
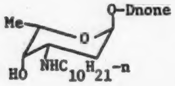
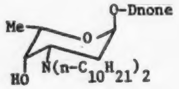
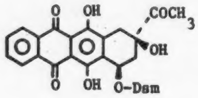
Compounds ***26*** and ***27*** are simply the hydrolytic cleavage fragments of DNR, daunomycinone ***[26]*** and daunosamine (the latter as the methylglycoside ***[27]***). In addition to their antitumor inactivity, neither component was especially toxic. Compound ***28*** is the daunosamine glycoside of a simplified synthetic aglycone in which the 4-methoxy and the 9-hydroxy and 9-acetyl moieties have

TABLE 3.—Inactive ADR/DNR analogs

Compound No.	Structure*	Tumor*	Toxic dose† (mg/kg)	Highest nontoxic dose (mg/kg)	Ref No.
26	 (daunomycinone)	L1210 (every day, Days 1, 5, and 9)	> 160	160	‡
27		L1210	400	200	‡
28	 1:1 diastereomeric mixture	P388 (every day, Days 1–9)	32	16	6
29		P388 (every day, Days 1–9)	16	8	§

See footnotes at end of table.

TABLE 3.—Inactive ADR/DNR analogs—Continued

Compound No.	Structure*	Tumor*	Toxic dose† (mg/kg)	Highest nontoxic dose (mg/kg)	Ref No.
30		P388 (every day, Days 1–9)	16	8	§
31		P388 (every day, Days 1–9)	> 200	200	¶
32	 (1:1 diastereomeric mixture)	P388 (every day, Days 1–9)	> 50	50	**
33		P388 (every day, Days 1–9)	12.5	6.25	††
34		P388 (every day, Days 1–9)	25	12.5	††
35		L1210	> 8	8	5‡‡

**See* footnotes * and † in table 1.

†Lowest dose causing early deaths in treated animals relative to the time of death of untreated tumor-bearing controls.

‡Unpublished NCI data.

§Wu H, Lee WW, Martinez A, et al. Unpublished work.

¶Wu H, Pickering M, Smith T, et al. Unpublished work.

**Fujiwara A, Stankorb J, Acton E, et al. Unpublished work.

††*See* footnote ** in table 2.

‡‡This compound [*35*] is the α-daunosaminide of the enantiomer of 4-desmethoxy-daunomycinone; its inactivity at 8 mg/kg should be compared with the potent activity of 4-desmethoxy-DNR at 0.5 mg/kg (*see* footnote ‡‡ in table 2).

been deleted. Since loss of methoxy is an activity-enhancing change, and it has been reported that 9-desacetyl-DNR is active as an antitumor agent,[4] the tentative conclusion may be reached that the 9-hydroxy is necessary for in vivo antitumor activity among the anthracyclines. It is significant that compound *28* retains substantial toxicity despite its inactivity. Thus, it appears that the simplifications leading to compound *28* don't cause decreased cytotoxicity but simply decreased specificity for tumor cells over normal host cells. Compound *28* is a mixture of diastereomers that were separated only on a small scale. The tentative in vivo properties of the individual diastereomers were consistent with the previously discussed properties of the mixture. Compounds *29* and *30* are the two diastereomers of another daunosamine glycoside obtained from a second simplified aglycone with the hydroxyl on the equivalent of the 8-position of DNR. Despite their in vivo inactivity, both compounds were quite toxic, again suggesting that the molecules lacked specificity but not intrinsic cytotoxicity.

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[4] Arcamone F. Antitumor anthracyclines. Presented at the 2nd Joint Canadian Institute of Chemistry-American Chemical Society Conference, Montreal, May 1977 (MEDI 22).

Deletion of most of the saturated ring from DNR, as in compound ***31***, resulted in loss of toxicity and antitumor activity. Deletion of all of the aromatic portions of the aglycone, leaving only the saturated ring and its attached daunosamine side chain ***[32]***, resulted in a biologically inert compound in vivo (at the limited doses available).

Although *N*-methylation of DNR provided a highly potent and efficacious derivative and *N*-benzylation provided a compound with enhanced efficacy but decreased potency, attachment of one or two *N*-decyl moieties to the nitrogen of DNR provided inactive and toxic derivatives. Since the lipophilicity added by the single decyl group of compound ***33*** is about equivalent to that of the two benzyl groups of compound ***21*** in table 2, the loss of activity must lie in the aliphatic character of the decyl group. The fact that both the mono- and di-decyl compounds ***[33*** and ***34]*** retain substantial toxicity, again suggests that these compounds lack selectivity, rather than innate cytotoxicity, for tumor cells.

The last entry in table 3, compound ***35***, illustrates a stereochemical point, inversion of both the 7- and 9-positions of 4-desmethoxy-DNR. These changes result in a compound showing no activity or toxicity at a dose 16 times greater than that of the isomer with normal aglycone stereochemistry.

DISCUSSION AND CONCLUSIONS

What generalizations can be made after this brief excursion into structure-activity relationships of ADR and DNR analogs? The first, and perhaps the most obvious, conclusion is that among the analogs examined to date, those possessing efficacy and potency comparable to, or superior to, parent antibiotics are quite similar to the parents in structural terms (ie, their hydrophilic character, functional groups, and stereochemistry). Ester ***6***, a possible exception, would certainly be much more lipophilic than ADR, but because of its ready hydrolysis in vivo it is perhaps best considered as a prodrug of ADR. The electronic properties of all compounds in table 1, ie, properties dictated by the chromophore, are very nearly identical with the exception of 5-imino-DNR ***[7]***. There has been comparatively little exploration of the chromophore to date because of difficult synthetic accessibility but it is intriguing that compound ***7*** and aclacinomycin A ***[3]***, which have appreciably altered electronic properties, are both reported to have improved cardiotoxic characteristics.

The second general conclusion is that high efficacy accompanied by decreased potency is more compatible with a much wider array of structural variants than is high efficacy accompanied by high potency. This is perhaps not surprising in view of the expectation that wider variations in chemical character would alter drug distribution properties more greatly, thus requiring that more drug be administered for an appropriate amount to reach the receptor site. On the other hand, this interpretation may be oversimplified in view of the types of variations compatible with activity. For example, an unusually wide range of ionic character is compatible with activity. Compound ***17*** must exist at physiologic pH as at least a dication, as probably does compound ***16***. The parent drugs are mono-cations and at least one very efficacious derivative, compound ***20***, is a neutral molecule. At physiologic pH, compound ***18*** would be a zwitterion bearing a negative charge in the region where other active analogs ***[16*** and ***17]*** bear positive charges or are neutral. Lipophilicity among highly efficacious analogs varies greatly as well. Dibenzyl analog ***21*** must be at least 4 orders of magnitude more lipophilic than DNR while glucosamine analogs ***22*** and ***23*** would be expected to be about 2 orders of magnitude less lipophilic than the parent antibiotics. An attractive interpretation of these phenomena is that the anthracycline antibiotics are operating by two or more mechanisms of action and that major changes in molecular character decrease involvement of a particular analog with receptors associated with one mechanism of action but do not affect (or possibly enhance) that molecule's involvement with receptors associated with other mechanisms. Biochemical work on ADR and DNR has increasingly indicated that multiple mechanisms may be involved with the antitumor action of these molecules. For example, it is well-established that nucleic acid metabolism is disturbed by the anthracyclines (3,6) but it has also been demonstrated more recently that mitochondria (17,18), cell membranes (19,20), microsomal electron transport (21,22), and tubulin binding (23) may also be involved in anthracycline antitumor action.

At a more chemical level, examination of data from tables 1–3 suggests that the sugar side chain is appreciably more susceptible to activity-retaining structural changes than is the aglycone, both in terms of functional groups and stereochemistry. With the exception of 9-acyl moiety alterations, significant changes in the aglycone usually resulted in inactive compounds.

While structure-activity studies among the anthracyclines have yet to reveal the ideal antitumor drug, accumulating information is consistent with the hope that improved antitumor action at the clinical level will be found among new analogs and that reduction of cardiotoxicity without loss of antitumor efficacy is feasible.

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Experimental Animal Models of Adriamycin Cardiotoxicity

James H. Doroshow,* Gershon Y. Locker, and Charles E. Myers[1]

SUMMARY

Since patients exhibit both acute and chronic cardiac toxic effects from Adriamycin, the salient characteristics of the reported animal models for both acute and chronic cardiotoxicity are reviewed. This review reveals that while the pathology of these models is often well-characterized, the physiologic, biochemical, and nutritional variables are, in general, poorly defined.

[Cancer Treat Rep 63:855–860, 1979]

Adriamycin (ADR), an anthracycline antibiotic, is one of the most useful drugs in oncologic practice (1). Its spectrum of antitumor activity extends from hematologic malignancies to breast and thyroid carcinomas as well as bone and soft tissue sarcomas. Unfortunately, dose-limiting cardiac pathologic lesions regularly occur in patients receiving ADR, and they may lead to a progressive, often fatal congestive cardiomyopathy (2).

The synthesis of new anthracycline analogs of unknown cardiotoxic potential, as well as recent investigations into the pathogenesis of ADR cardiotoxicity (3–5), has led to the development of several animal models of this syndrome (table 1).

The clinical characteristics of human anthracycline-induced cardiac damage have recently been reviewed (6). This cardiomyopathy is characterized by evidence of acute and chronic damage. The acute toxicity appears within hours or days of ADR administration and is manifested as electrocardiographic (ECG) changes and the appearance of abnormal left ventricular function (7). In occasional patients this acute toxicity may be sufficient to manifest as congestive heart failure (CHF), and this syndrome has been termed the pericarditis-myocarditis syndrome (8).

Pathologic evidence of the chronic cardiomyopathy appears early in patients receiving ADR therapy (2). Furthermore, the magnitude of the pathologic change observed is a function of the total cumulative dose of ADR administered. Clinically evident CHF is thus a late manifestation of steadily accumulating subclinical cardiac damage. Most of the experience with human ADR cardiotoxic reactions has been in patients who received 45–75 mg/m^2 of the drug every 3–4 weeks, although data have recently been accumulated on the properties of a weekly schedule of drug administration (9).

It is unclear at present whether the acute and chronic cardiotoxicity are related. It is important to remember that the anthracyclines are complex in their actions. They have been shown to intercalate DNA (10), bind directly to cardiolipin (11), chelate physiologically important metal ions (12), cause superoxide radical generation (3,4), inhibit ubiquinone-dependent enzymes (13), and inhibit cardiac metmyoglobin reductase (14). This is, in itself, an incomplete list. So it is entirely possible that the acute and chronic cardiotoxicity might have different pathogeneses. If this is true, then it is also possible that there may be major interspecies variations in the susceptibility to acute versus chronic cardiotoxicity. The same pattern of thinking can be applied to the development of anthracycline analogs or the development of antidotal agents. The implications are that no assumptions should be made a priori about the relationship of acute to chronic anthracycline-induced cardiomyopathy.

In fact, we have found no evidence in man which convincingly shows whether the acute and chronic toxic effects are pathogenetically linked or not. At present, two distinct hypotheses seem equally tenable. One hypothesis states that there is a dose-dependent wave of myocardial damage which occurs after ADR administration and which is followed by recovery. If that dose is high enough, the recovery may become incomplete as a portion of the muscle cells undergo irreversible damage. This could provide an explanation for the cumulative nature of the pathologic damage as seen in the endocardial biopsy. On the other hand, acute and chronic toxic effects may be unrelated events. For these reasons, we have tried in the discussion which follows to distinguish between animal model work which relates to acute cardiotoxicity versus chronic cardiotoxicity.

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Table 1 represents a compendium of animal model studies (15–32). We have attempted to make it as complete as possible and apologize to anyone whose contribution was inadvertently excluded. It is apparent that most work has centered on three animal species: the rabbit, the rat, and the mouse.

The rabbit represents the most completely described model of chronic cardiomyopathy. Using a variety of treatment protocols, numerous laboratories have reported the development of cardiac lesions identical to those described in man which include vacuolation, edema, and myofibrillar disruption. The rabbit model does appear to differ from man in that a treatment schedule of small weekly doses (2 mg/kg/week) creates dramatic cardiac damage in the rabbit but apparently not in man (9).

Work done in the Laboratory of Toxicology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, has also shown that certain biochemical changes occur during the development of cardiomyopathy in rabbits, including an increase in myocardial calcium content and the appearance of creatinine phosphokinase (CPK) in the blood.

The rabbit model has several problems which may be limiting under certain circumstances. First, cost may be a problem; compared with the other species to be discussed, the rabbit is more expensive to purchase, house, and feed. Because of the rabbit's size, more drug is required. This is a particular problem in analog screening where compounds may only be available in small amounts and at a high cost. Second, a selection of isogenic inbred strains are not available at present. The investigator must thus always consider and control for possible important genetic differences in rabbits from different sources or even genetic variation within rabbits from a single source. Third, well-characterized transplantable tumors are not available. This means that anthracyclines compared for cardiac toxicity in the rabbit can not be tested for antitumor effect in the same model. This problem becomes most significant in the screening of analogs where the major goal is an effective ranking of compounds according to their toxic/therapeutic ratio. Here the presence of major interspecies differences in drug metabolism or distribution could have significant effects on analog ranking. While such problems are presently largely theoretic, they can be avoided by using other available models.

The rat model, while less widely used, appears to have all of the advantages of the rabbit model without many of its disadvantages. Mettler et al (22) have shown that the rat develops clinical and pathologic evidence of chronic cardiomyopathy similar in its characteristics to cardiotoxicity in man and rabbits. This work has been extended by Olson and Capen (24,25), who have carefully studied both acute and chronic cardiomyopathies within the same inbred strain of rat. They have shown that large single doses or multiple doses administered within a short period of time cause rapid development of extensive cardiac damage characterized by dilation of the sarcoplasmic reticulum, nuclear pyknosis, focal myocyte degeneration, and mitochondrial swelling followed by degeneration. These mitochondrial changes, while a significant part of the acute syndrome, have been described as not being characteristic of the chronic cardiomyopathy in this species. Repeated smaller doses (2 mg/kg × 7 days) result in the development of chronic cardiomyopathy and CHF.

The calcium ion shifts and elevation in serum CPK reported in the rabbit occur also in the rat. Of even greater interest is that they occur roughly in parallel with the pathologic changes in both the acute and chronic cardiotoxic versions of this model.

In perhaps a related development, Bachmann and coworkers (21) have shown that a variety of anthracyclines cause dramatic dose-related changes in the QRS complex in the ECG. This observation provides the basis for a rapid and economical screen of anthracyclines.

As an added advantage, the rat is available in a variety of genetically homogenous strains and offers a range of transplantable tumor lines. Its size is large enough to make iv injections practical and its heart size is sufficient to make it convenient for both biochemical and physiologic studies. Finally, compared to the rabbit, it is less expensive to buy, house, and feed.

The acute cardiotoxicity of ADR in the mouse has been described in several laboratories (5,26,27,31). The most complete description is that of Lambertenghi-Deliliers et al (32). They describe the early appearance of nucleolonema fragmentation after a single iv dose of 10 mg/kg of ADR, followed by the appearance of swollen damaged mitochondria, enlargement of the smooth endoplasmic reticulum, and cisternal and myofibrillar degeneration. Fifty days after these acute changes, extensive chronic changes were evident; these changes occurred without the need for additional drug administration.

Chronic cardiomyopathy apparently does develop in mice; however, at present it is only briefly described in one paper and one abstract (28,31). From the material reported, the cardiac lesions seen are morphologically similar to those in man or rabbit but seem to differ in distribution with the atria being more commonly affected in mice and the ventricles more affected in man, rabbits, and rats.

In its favor, the mouse is available in a wide range of inbred strains for which a large number of well-characterized transplantable tumors are available. It is the least expensive of the three species in terms of purchase price, housing, and drug requirements. In addition, the National Cancer Institute and other institutions have had

TABLE 1.—Experimental models of ADR cardiotoxicity

Species (ref No.)	Drug	Route of administration	Treatment schedule	Immediate (I) vs delayed (D) cardiac toxicity	Markers of cardiac toxicity	Study conclusions
Rhesus monkey (15)	ADR	iv	Single doses of 3, 4.5, 6, 7.5, and 9 mg/kg; monthly doses of 3, 4.5, and 6 mg/kg ± cyclophosphamide	D	Clinical features of CHF; light microscopy	Severity of lesions in striated muscle dose-dependent
Rabbit						
New Zealand white (NZW), pathogen-free, male (16)	ADR	iv	7.7 mg/m^2 (0.7 mg/kg) every other day to a total of from 200 to 400 mg/m^2	D	CHF; light and electron microscopy; cardiac muscle Na^+, Ca^{++}, and H_2O; serum CPK, LDH, and electrolytes	Clinical CHF correlates with morphologic abnormalities that include vacuolation, edema, myofibrillar disruption, and mitochondrial swelling
NZW, male and female (17)	ADR	iv	2.25 mg/kg/wk in 3 divided doses to a total of 85–270 mg/m^2	D	CHF; hematocrit; total wbc count; light and electron microscopy; serum CPK and LDH	Pathologic lesions progressed after drug stopped
NZW, male (18)	ADR	iv	1.2 mg/kg twice/wk for 9 wks ± 8.5 mg/kg of α-tocopherol im and 0.03 mg/kg of sodium selenite 24 hrs before each ADR dose	D	CHF; wbc; Hgb; BUN; CPK; light and electron microscopy; body weight; survival time	Selenium-α-tocopherol treatment lessens histologic evidence of cardiac toxicity
NZW (19)	ADR	iv	100 mg/m^2 single dose ± 200 mg of α-tocopherol im daily × 4 days before ADR treatment	I	ECG; rbc; glutathione	α-Tocopherol pretreatment resulted in increased animal survival, reduced ECG abnormalities, and ablation of rbc glutathione drop
Rat						
Wistar (20)	Daunorubicin	iv	Single dose of 25 mg/kg	I	Light and electron microscopy	Maximal electron microscopic changes consisting of membranous whorls, edema, mitochondrial swelling, and degeneration 5 days after dose
Siv-50, female (21)	ADR and daunorubicin	ip	1, 4, 8 mg/kg × 5 doses/wk	I	ECG; mitochondrial respiration	Characteristic ECG findings dose-related; altered mitochondrial oxygen consumption but no change in oxidative phosphorylation by ADR
CDF, male (22)	ADR	iv and sc	1–2 mg/kg/wk × 10–14 wks	D	CHF; survival; light and electron microscopy; body weight; serum Ca^{++}; BUN and CPK	Consistent development of clinical and pathologic cardiac toxicity with characteristic ultrastructural features
Brown-Norway (23)	ADR	iv	7.5 mg/kg on Day 1 and 10 mg/kg on Day 15, ± pretreatment with 2375 mg/kg of α-tocopherol	D	ECG; total body and heart weight; light microscopy	α-Tocopherol partially prevented ADR-induced weight loss, ECG changes, CHF, as well as cardiac pathologic abnormalities

TABLE 1.—Experimental models of ADR cardiotoxicity—continued

Species (ref No.)	Drug	Route of administration	Treatment schedule	Immediate (I) vs delayed (D) cardiac toxicity	Markers of cardiac toxicity	Study conclusions
NZB-Pl/Cr (24,25)	ADR	iv, sc, and ip	20 mg/kg × 1 dose; 13 mg/kg; 10 mg/kg × 1 or 2 doses; 5 mg/kg × 1 or 2 doses; 1 or 2 mg/kg every 7 days × 10 doses	I and D	CHF; body weight; serum and cardiac Ca^{++}; CPK and LDH	High doses produce swelling and degeneration of mitochondria, dilation of sarcoplasmic reticulum, and myofibrillar lesions; repeated low doses produce consistent CHF and cardiomyopathy
Mouse						
BDF$_1$, female (26,27)	ADR	ip	10 and 20 mg/kg	I	Myocardial; DNA synthesis; light and electron microscopy	Ultrastructural abnormalities including vacuolization, edema, mitochondrial swelling, myelin whorls, and disorganization of the sarcotubular apparatus maximal 6 days after dose; suppression of cardiac DNA synthesis persisting for 5 days after ADR
Swiss CD-1, male (28)	ADR	iv	6.7 or 10 mg/kg twice/wk to a total dose of 20, 30, or 33.5 mg/kg, or 20 mg/kg (single dose)	D	Light and electron microscopy; CHF	Characteristic light microscopy lesions seen 8–12 wks after the end of treatment; degree of cardiomyopathy related to total ADR dose; atrial lesions prominent
CDF$_1$, male (5, 29, 30, 31)	ADR	ip	5, 10, 15, or 20 mg/kg (single dose) ± α-tocopherol pretreatment	I and D	Light and electron microscopy; heart weight; survival; malondialdehyde formation; cardiac glutathione peroxidase activity	α-Tocopherol changes the dose-response curve of ADR while blunting markers of cardiac lipid peroxidation without inhibiting antitumor response; ADR toxicity is enhanced by a deficiency of cardiac glutathione peroxidase
CRF, male (32)	ADR	iv	10 mg/kg (single dose)	I and D	Electron microscopy	Early, sequential appearance of nucleolonema fragmentation, damaged mitochondria, enlarged smooth endoplasmic reticulum cisternae, and myofibrillar degeneration; by 50 days, extensive damage visible

extensive experience in the use of the mouse and its transplantable tumor lines as a drug screen. It is for these reasons that we suggest that greater effort be placed on characterizing the mouse as a model for chronic anthracycline cardiotoxicity.

GENERAL COMMENTS

There is evidence that diet may significantly affect the development of cardiomyopathy in each of these species. In 1975 Handa and Sato (3) showed that daunorubicin stimulated superoxide production in liver microsomes. Other investigators have shown that metabolic reduction of ADR and daunorubicin to their respective semiquinone radicals is followed by subsequent reduction of molecular oxygen to superoxide. This has been shown to occur in liver microsomes, cardiac sarcomas, cardiac mitochondria, human rbcs and platelets, and intact Ehrlich ascites tumor cells (3–5,33–38). The toxic effects of the superoxide radical are well-described (39) and appear to play a role in such diverse phenomena as phagocytic killing of ingested bacteria, radiation damage, oxygen toxicity, and paraquat poisoning. Baseline levels of superoxide production are associated with a variety of normal metabolic processes. It is therefore not surprising that most mammalian cells are equipped with elaborate defenses against superoxide damage. The essential components of this system are the enzymes superoxide dismutase, catalase and glutathione peroxidase, and α-tocopherol.

While the full biologic consequences of anthracycline-induced superoxide production are far from clear, it has been postulated that it plays a role in the development of the cardiotoxicity (5). Our laboratory has detected evidence of lipid peroxidation in mouse hearts following treatment with ADR and reported that administration of large doses of α-tocopherol not only suppressed lipid peroxidation but lessened the degree of morphologic damage seen in this acute cardiotoxicity model. These observations have recently been duplicated in rats (23). In both of these studies, the doses of α-tocopherol used were very large and outside the range of normal dietary variation. In addition, both studies focused on acute rather than chronic cardiomyopathy. It would be more interesting and more pertinent to determine if normal dietary levels of α-tocopherol and other anti-oxidants affect the development of chronic cardiomyopathy.

There is one study which addresses itself to this problem (18). These authors showed that regular administration of a commercially available α-tocopherol-selenium mixture lessened the magnitude of the ADR-induced cardiomyopathy in the rabbit. It was impossible to determine whether the effect seen resulted from the administration of α-tocopherol, selenium, or the combination. The potential importance of selenium is suggested by a number of observations. First, in most mammals, tissue concentration of the key free-radical defense enzyme, glutathione peroxidase, is directly determined by the selenium content of the diet (40). Second, work from our laboratory has indicated that the dietary level of selenium has a major impact on the whole-animal toxicity of ADR (6). Third, other investigators have been able to show that the selenium content of grain and other common ingredients in commercial animal chow can vary from deficient to toxic (41).

None of the animal model studies reviewed here report any effort to control α-tocopherol, selenium, or any of the other nutritional factors known to affect susceptibility to free-radical damage (42). Standardization of dietary levels of α-tocopherol and selenium would seem prudent, at least until the role of these factors in the development of both acute and chronic cardiomyopathy has been settled.

As we have shown, acute cardiotoxicity after ADR has been described in man (7), rats (21–24), mice (5,25,27,29,31), and rabbits (13). In this context, the observation of Lambertenghi-Deliliers et al is interesting (32). They report that CRF mice given a single iv dose of 10 mg/kg of ADR develop well-defined acute cardiac pathologic changes followed in 50 days by diffuse chronic cardiomyopathy. In addition, as we have previously discussed, Van Vleet et al (18) have reported that selenium and vitamin E, agents we and others (5,19,23,29) have shown to lessen acute toxicity, also may affect chronic cardiomyopathy.

These two studies suggest that the acute and chronic cardiotoxicity may be viewed as acute and delayed expression of free-radical damage to the same tissue. There is an analogy to this in the acute and delayed effects of radiation damage, a known free-radical process. Further work is needed to determine whether this hypothesis is viable. The major attraction of this hypothesis is that it provides the hope that circumventing the initial free-radical process may prevent both acute and delayed toxic effects. Unfortunately, even though radical scavengers have been shown not to alter the antitumor effect of ADR in selected mouse and rat tumors (5,23), there is no guarantee that a similar differential in the responsiveness of human tumors will be found.

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Pharmacokinetic, Toxicologic, and Chemotherapeutic Properties of Detorubicin in Mice: A Comparative Study With Daunorubicin and Adriamycin[1]

D. Deprez-de Campeneere,* R. Baurain, and A. Trouet[2,3]

SUMMARY

We have studied the stability, pharmacology, toxicology, and therapeutic activity in mice of detorubicin, a new semisynthetic derivative of daunorubicin. In vitro, detorubicin remains stable under acidic conditions while it is very quickly hydrolyzed into Adriamycin under neutral pH conditions. In vivo, the hydrolysis of detorubicin into Adriamycin occurs in the bloodstream a few minutes after iv injection. The tissue distribution of detorubicin in mice is, however, very distinct from that observed after administration of Adriamycin and daunorubicin. The therapeutic effect of detorubicin on the sc implanted L1210 leukemia is superior to that of daunorubicin and at least equal to that of Adriamycin. Detorubicin can thus be considered a prodrug of Adriamycin with very distinct pharmacokinetic and perhaps therapeutic properties.

[Cancer Treat Rep 63:861–867, 1979]

Detorubicin (14-diethoxyacetoxydaunorubicin; DET) is a new semisynthetic anthracycline derivative described by Maral et al (1). This compound exhibits interesting activity against several experimental tumors. The first clinical trials, performed by Jacquillat et al (2,3), indicated that DET seems to be a promising drug. We have undertaken a detailed in vivo study of DET in mice after iv administration and have compared the pharmacologic, toxicologic, and therapeutic properties of this new drug with those of daunorubicin (DNR) and Adriamycin (ADR).

MATERIALS AND METHODS

DET and DNR were provided as hydrochlorides by Rhône-Poulenc (Paris, France). DNA from herring sperm (type VII) was purchased from Sigma Chemical Co (St. Louis, Mo). Drugs and their fluorescent metabolites were analyzed by high-pressure liquid chromatography (HPLC) according to the method described previously for ADR by Baurain et al (4).

Two mouse strains were used: NMRI (Proefdierencentrum, Heverlee, Belgium) and DBA_2 (Charles River, St. Aubin-lès-Elbeuf, France). Drugs were administered to female mice (average weight, 22 g) in the tail vein. The determinations of plasma and tissue levels were performed using NMRI mice according to methods previously described (5).

The toxicity studies were done using NMRI mice. The drugs were given iv on 2 consecutive days and the LD50 was calculated after 30 days of observation by log-probit regression of the data obtained with at least four different doses per drug and an average of ten mice for each dose.

The chemotherapeutic activity of the drugs was evaluated in L1210 murine leukemia (5). The L1210 cells were inoculated iv or sc into DBA_2 mice. In both cases the drugs were administered iv on Days 1 and 2 following the inoculation of cells. The chemotherapeutic results are expressed in terms of increase in lifespan and of animals surviving 30 days. For the sc inoculated form of L1210 leukemia the progression of the tumor was followed by daily palpation and estimation of the average tumor diameter with calipers.

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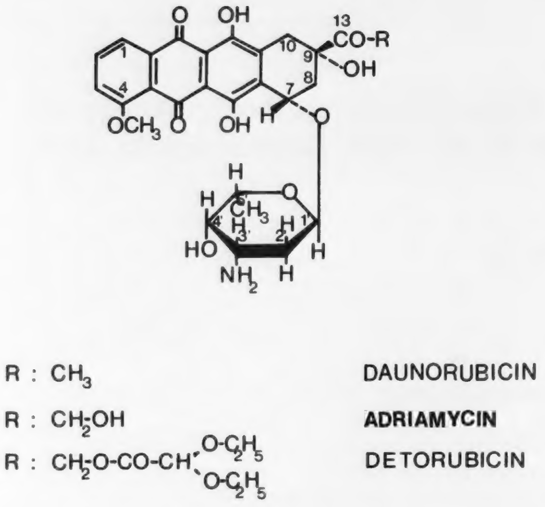

FIGURE 1.—Structures of DNR, ADR, and DET.

RESULTS AND DISCUSSION

The chemical structure of DET is shown in figure 1; it is a derivative of DNR possessing an ester function in the C_{14} position.

It is possible to separate and quantitate DET, ADR, and DNR by HPLC and fluorometry as shown in figure 2. The retention times are 2.2 minutes, 3.1 minutes, and 4.8 minutes for DET, DNR, and ADR respectively at a flow rate of 1.2 ml/minute.

DET hydrolyzes and generates ADR; this hydrolysis, as illustrated in figure 3, depends on the pH and temperature of the solution. When dissolved at a final concentration of 250 μg/ml and incubated at 22°C (fig 3A), DET is very stable at pH 4.5 (acetate buffer, 0.5 M); however, it hydrolyzes very rapidly into ADR at pH 7.4 (phosphate buffered saline). The half-life values of hydrolysis (time needed to obtain 50% hydrolysis of DET) are 10 hours at pH 4.5 and 5.4 minutes at pH 7.4. When dissolved in twice-distilled water at the same concentration, an intermediate stability is obtained with a pH value of 5.8. As illustrated in figure 3B, when dissolved in serum at 250 μg/ml (pH ± 7.7), DET is even more unstable at 37°C with a half-life of 4.6 minutes; this hydrolysis rate is decreased when the incubation is performed at 0°C (half-life = 16.7 minutes). We observed that the stability of DET can be enhanced by combining the drug with high molecular weight DNA at a nucleotide:drug molar ratio of 20. Under these conditions, the half-life of hydrolysis is 57 minutes in the presence of calf serum at 37°C and 522 minutes at 0°C (fig 3B).

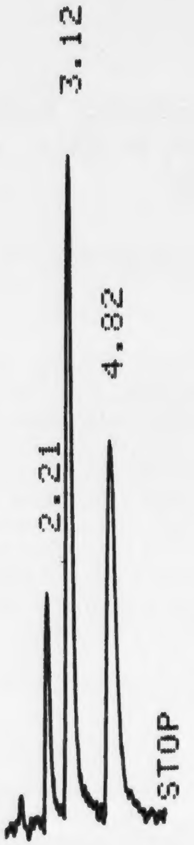

FIGURE 2.—Separation of DET, DNR, and ADR by HPLC. The drugs were separated from a mixture by HPLC and estimated by fluorometry as described by Baurain et al (4). The following retention times were obtained: 2.21 mins (DET), 3.12 mins (DNR), and 4.82 mins (ADR) at a flow rate of 1.2 ml/min.

These results have important practical implications when using DET for experimental or clinical purposes since it is essential to keep the solutions cold for the shortest time possible before administration. In our experiments, solutions were prepared immediately before use and kept at 0°C. By the end of the injection in animals, we observed by HPLC that the solutions of DET contained < 6% of ADR. To minimize the hydrolysis of DET in the plasma during pharmacologic experiments, we collected the blood samples on DNA (1.17 mg) and these were analyzed immediately.

The plasma levels of DET and of its hydrolysis product ADR were determined in NMRI mice after an iv injection of 8.75 mg/kg of DET (this dose corresponds to 7 mg/kg of DNR on an equimolar basis). As illustrated in figure 4, DET disappears very rapidly from the plasma while

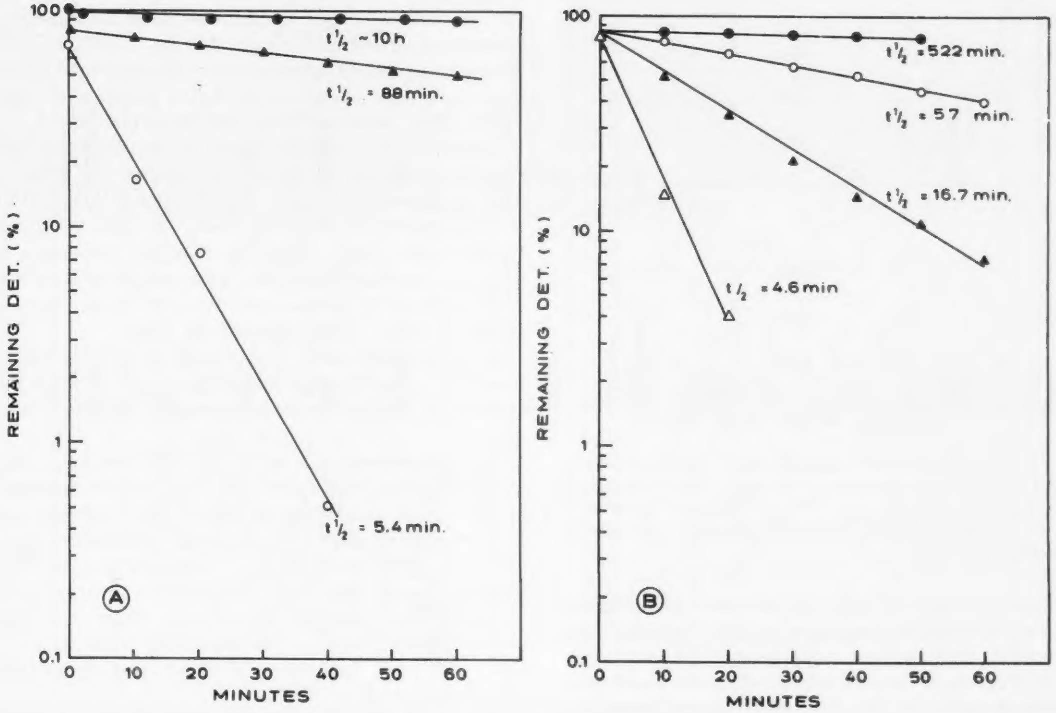

FIGURE 3.—Stability of DET in solution. DET solutions at 250 μg/ml were prepared under different conditions. After various times, the amount of DET still present in the solutions was determined by HPLC and expressed as % of remaining DET. (A) Stability at 22°C in acetate buffer (0.5 M, pH 4.5) (●), twice-distilled water (pH 5.8) (▲), and phosphate buffered saline (pH 7.4) (○). (B) Stability in calf serum (pH ± 7.7) in presence or absence of DNA (nucleotide:drug molar ratio = 20). DET at 37°C (△) and at 0°C (▲); DET-DNA at 37°C (○) and at 0°C (●).

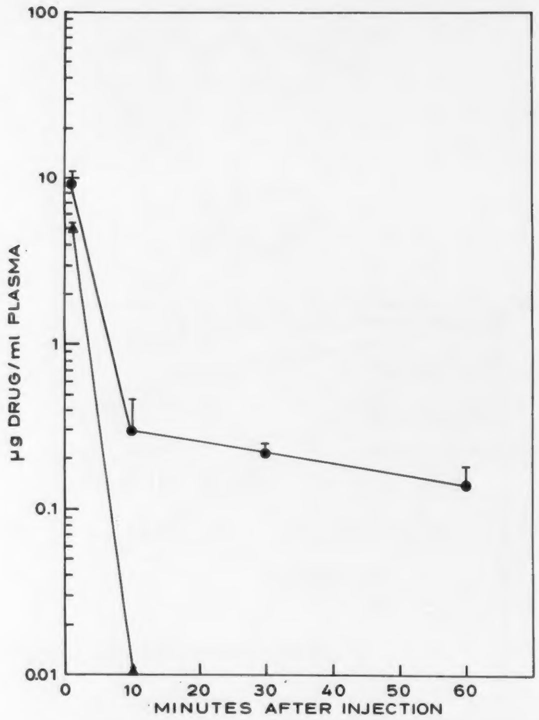

FIGURE 4.—Plasma levels of DET and ADR after iv injection of DET in NMRI mice. DET was injected iv at 8.75 mg/kg. After various times, the individual blood samples were collected on DNA. DET (▲) and ADR (●) were detected in the plasma by HPLC. Means + SD of 3 separate experiments are shown.

significant amounts of ADR can be detected 1 minute after the injection. It is important to point out that when the blood samples are not collected on DNA, six times less DET can be detected 1 minute after administration. Ten minutes after iv injection, DET can no longer be detected in the plasma and only ADR is still present, disappearing from the plasma with an estimated half-life of 45 minutes. The plasma half-life of DET plus ADR corresponding to the first elimination phase equals 1.8 minutes as compared to 0.9 minute for ADR and 1.4 minutes for DNR (4,5).

The tissue concentrations of DET, ADR, doxorubicinol, and aglycones, obtained in various tissues 30 and 120 minutes after iv injection of 8.75 mg/kg of DET, are illustrated in figure 5. From these results it is obvious that ADR is the primary compound concentrating in tissues and is found in total blood, bile, and urine after injection of DET. DET is found in appreciable amounts only in the heart after 30 minutes and in the colon after 2 hours. After 30 minutes, DET and its metabolites accumulate in the following tissues (in order of decreasing concentrations): kidney, heart, liver, lung, duodenum, colon, spleen, stomach, and brain. After 120 minutes, the drug levels remain high in the kidney, increase in the lung, duodenum, colon, and stomach, and decrease in the liver and heart. No appreciable amounts of doxorubicinol could be found; the same was true for aglycones, except in the colon.

The tissue distribution of anthracyclines obtained after administration of DET is significantly different from that obtained after injection of DNR and ADR. This is very clear from figure 6, which illustrates the total amount of drugs (sum of parent drugs and metabolites) found in various tissues 30 and 120 minutes after the iv administration of an equimolar dose of DNR, ADR, or DET. Since the amount of aglycones is very small in all cases, these data refer mainly to the active compounds DET, ADR, DNR, daunorubicinol, and doxorubicinol.

Except for the heart and the liver, significantly lower drug levels (parent drug plus metabolites) are found in various tissues 30 minutes after administration of DET in comparison with DNR and ADR. The urinary concentration is also lower. After 120 minutes, differences in tissue accumulation decrease when comparing DET and ADR, although there is an indication of a higher concentration of DET at that time in the colon.

As indicated in table 1, the LD50 of DET is higher than that of ADR and close to that of DNR when the drugs were injected iv on 2 consecutive days to NMRI mice.

The chemotherapeutic activity of DET has been compared to that of ADR and DNR using L1210 leukemia in DBA_2 mice. Against the iv form of L1210 leukemia and after iv administration of the drugs on 2 consecutive days, DNR and ADR were found to be equally active and DET was significantly less active, especially in terms of long-term survivors (table 2). In contrast, when the L1210 cells were inoculated sc and the drugs were given iv, DET exerted a therapeutic effect at least comparable with that of ADR (table 3).

CONCLUSIONS

We can conclude that in spite of its rapid transformation into ADR, DET possesses very distinct properties as compared to ADR and DNR when these drugs are administered under similar conditions. First, the tissue distribution of drugs and metabolites differs significantly after injection of DET compared to that obtained after administration of DNR and ADR. Secondly, DET has been found to be particularly active in sc implanted L1210 leukemia and the therapeutic effect obtained with DET is superior to that of DNR and at least equal to that of ADR. Finally, DET can be viewed as a prodrug of ADR, characterized however by distinct pharmacologic and perhaps therapeutic properties. More extensive studies on a variety of animal tumors are needed to confirm and extend our observations.

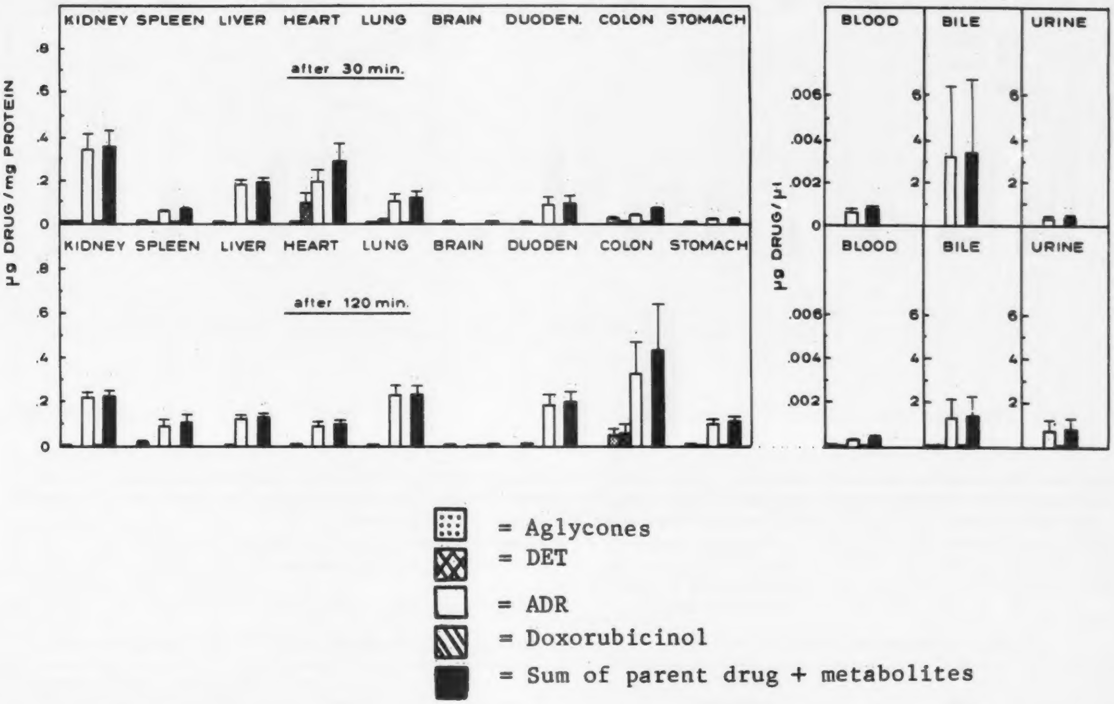

FIGURE 5.—Tissue distribution of aglycones, DET, ADR, and doxorubicinol 30 and 120 mins after iv injection of DET in NMRI mice. Drug concentrations were determined in several tissues, blood, bile, and urine 30 and 120 mins after iv injection of DET at 8.75 mg/kg. Means ± SE of 4 different experiments are shown.

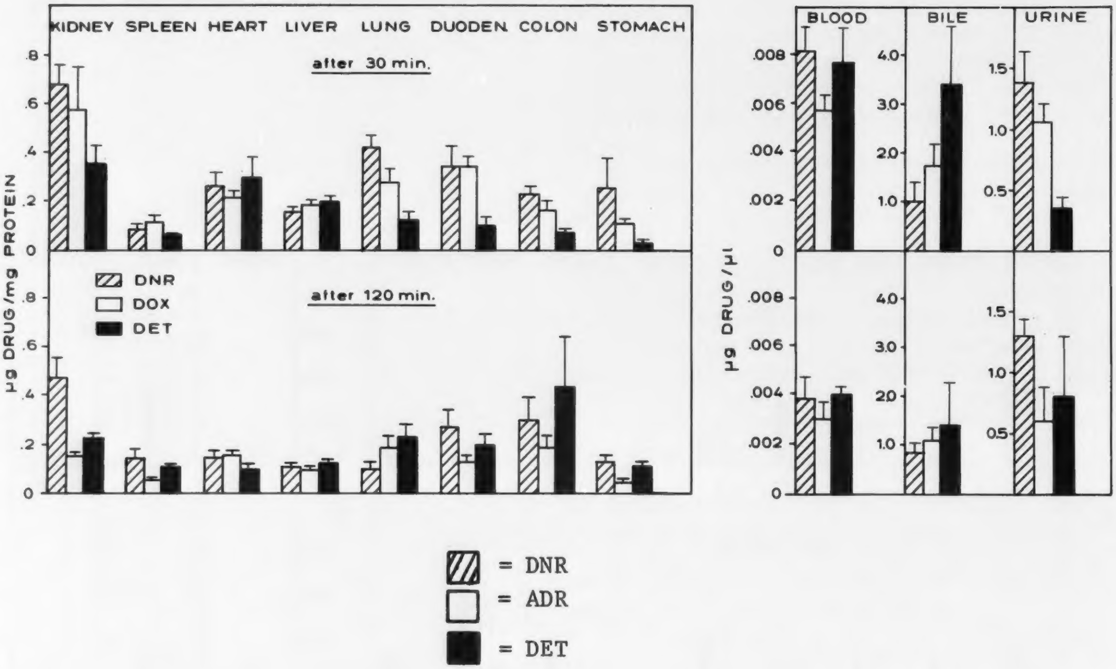

FIGURE 6.—Tissue distribution of DNR, ADR, DET, and their metabolites after iv injection in NMRI mice at equimolar doses. Drugs were given iv at equimolar doses, corresponding to 7 mg/kg for DNR. Mice were killed after 30 or 120 mins. Total amount of parent drug plus fluorescent metabolites after administration of DNR, ADR, or DET is given. Means ± SE of at least 4 individual experiments are shown.

TABLE 1.—Comparative toxicity in NMRI mice

Drug	LD50* (mg/kg/day)
DNR	15.5
ADR	11.3
DET	15.3

*LD50 = dose which induces 50% lethality in mice after 30 days of observation. Drugs were given iv for 2 consecutive days.

TABLE 2.—Chemotherapeutic activity in L1210 leukemia inoculated iv*

Drug	Dose (mg/kg/day)	Increase in lifespan (%)	No. of survivors on Day 30/ total No. of mice
DNR	10	42	0/18
	12	110	16/122
ADR	5	50	0/16
	6	61	0/16
	7	86	4/17
	8	108	4/28
DET	6	33	0/8
	7	41	0/9
	9	83	0/8
	12	83	0/8

*10^4 L1210 cells per mouse were inoculated iv on Day 0 into DBA_2 mice. Drugs were given iv on Days 1 and 2.

TABLE 3.—Chemotherapeutic activity in L1210 leukemia inoculated sc*

No. of L1210 cells	Drug	Dose (mg/kg/day)	Increase in lifespan (%)	No. of survivors on Day 30/ total No. of mice	Average tumor diameter (mm) on Day—	
					8	15
10^5	—	—	0	0/33	7.5	—
	DNR	10	55	0/17	0.3	4.5
		12	74	0/35	0	3.0
	ADR	6	142	4/9	0	0.9
		7	523	8/9	0	0
	DET	9	166	2/6	0	0
		10	360	4/7	0	0
2.5×10^5	—	—	0	0/13	9.9	—
	DNR	11	47	0/6	2.1	6.9
	ADR	6	83	0/8	0	1.2
	DET	6	45	0/9	2.7	5.7
		9	89	1/9	0	2.1
5×10^5	—	—	0	0/43	11.7	—
	DNR	10	44	0/9	4.5	7.5
		12	50	0/9	3.6	6.9
	ADR	6	61	0/36	2.4	6.8
	DET	6	31	0/8	4.5	9.0
		9	65	1/9	0	5.1

*L1210 cells were inoculated sc on Day 0 into DBA$_2$ mice. Drugs were given iv on Days 1 and 2.

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The Interval-Force Relationship: A Technique for Evaluating the Cardiac Toxicity of Anthracycline Analogs[1]

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With the technical assistance of D. de Moes[3]

SUMMARY

The most serious side effect of the anthracycline derivatives is dose-related cardiac toxicity induced during repeated administration. An in vitro method is described which assesses the interval-force relationship in evaluating the contractility of the rat and rabbit heart. Repeated administration of Adriamycin resulted in a progressive decrease in contractility which correlated closely with the cumulative dose administered. This model offers a reliable method to evaluate the effect of new anthracycline analogs on the heart and to study the potential of other drugs or agents to protect against cardiac toxicity.

[Cancer Treat Rep 63:869–873, 1979]

Although anthracycline antibiotics have proved to be among the most effective antitumor agents, the cardiac toxicity of this group of drugs remains a major obstacle to prolonged application by medical oncologists. An anthracycline derivative with antitumor activity but without a detrimental effect on the heart is still the main goal of investigators in the field of anthracycline research. The persistent search for nontoxic derivatives has provided > 500 analogs, each of which deserves adequate testing for antitumor activity and, if therapeutically active, evaluation of its effect on the heart. A method to assess the cardiotoxicity of anthracycline analogs in laboratory animals should be reliable and workable. The predictive value of the available techniques is controversial.

The mechanical function of the heart is to pump blood to allow the delivery of oxygen and the removal of waste products. Neither quantification of light- and electron-microscopic alterations nor electrophysiologic phenomena reflect the impairment of the mechanical activity of the heart after prolonged exposure to anthracyclines. There is a need for a reliable parameter for the contractile behavior of the heart (1). Unfortunately, no simple in vivo method can satisfactorily reflect myocardial contractility without being affected by the preload and/or afterload of the heart. Assessment of right and/or left ventricular function in intact animals given a myocardial depressant is therefore subject to some uncertainty (2).

Extracardiac factors such as the lungs, pericardium, and peripheral circulation can strongly influence cardiac pump function irrespective of myocardial contractility. In fact, anthracyclines do cause disturbances in body fluids, which means that variation in the preload and/or afterload of the left ventricle may occur (3).

The isolated heart perfused according to Langendorff is an accepted model for the study of cardiac effects of pharmacologic agents. It offers the advantages of stability and allows the exclusion of extracardiac factors. The apicobasal shortening of the preparation can be considered a parameter for contractility.

The known relationship between the heart rate and the intrinsic contractile behavior must be defined. Electrical stimulation of an isolated heart makes it possible to analyze the whole appropriate frequency range. The curve depicting the so-called interval-force relationship was first described by Kruta in 1937 (4).

This paper describes an interval-force relationship for the hearts of two animal species. All animals had been repeatedly exposed to Adriamycin up to a cumulative dose known to cause chronic cardiac toxicity in both the rabbit and the rat (1,5). The model consists of perfused isolated artificially stimulated hearts, avoids preload and afterload, and uses perfusates of standardized composition. This approach provides a reliable "endpoint parameter" (1) for the evaluation of the effects of anthracyclines on the contractile function of the heart.

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MATERIAL AND METHODS

Animals

Male New Zealand white rabbits weighing 2.9–3.9 kg were maintained in a climatized environment isolated from other animals. Rabbits were given water and pelleted rabbit food ad libitum. The food contained 89 mg of α-tocopherol per kg supplemented with α-tocopherol-acetate and 0.45 ppm of selenium. One week after a predetermined cumulative dose of Adriamycin (400 mg/m^2) had been reached, the animals were killed as described below.

Wistar rats weighing 190–220 g were kept in Perspex cages, four to a cage, and were given water and pelleted rat food ad libitum. After 6- and 12-week treatment periods, animals were randomly taken from the population and used for the assessment of contractile function. For each treatment period, four rats that did not receive Adriamycin served as controls.

Drug Administration

Fourteen male rabbits received Adriamycin at a dose of 2 mg/kg/week given in two injections into the marginal ear vein. The control rabbits received equal volumes of 0.9% NaCl solution iv twice a week.

Sixteen female Wistar rats were given Adriamycin via the tail vein at a dose of 2.0 mg/kg/week after being lightly anesthetized with ether. Before and after the injection the vein was flushed with 0.1 ml of 0.9% NaCl to avoid toxic necrosis of the tail. The seven control rats received equal volumes of 0.9% NaCl solution.

Preparation of the Animals

Prior to the perfusion of the isolated hearts the rabbits were anesthetized with hypnorm (1 ml/kg im) and the rats were anesthetized with ether. When sufficient anesthesia had been obtained, the animals were heparinized. The chest was then opened, the thymus dissected, and the aorta presented. Next the pericardium was opened and a ligature was placed around the aorta. A Perspex cannula was introduced into the aorta and the heart was excised from the pericardium and connected to the perfusion system via the cannula.

Perfusion System

The perfusion according to Langendorff's technique was performed with an apparatus developed by Zimmerman et al (6) (fig 1). Constant perfusion pressure is obtained by means of a water lock. For the rabbits a pressure of 80 cm H_2O was maintained and for the rats a pressure of 100 cm H_2O was maintained. The temperature of the perfusate was kept constant at 37.5°C. The composition of the perfusate (in mmols/liter) was sodium, 149; potassium, 8.4; calcium, 5.2; magnesium, 4.2; chloride, 138; HCO_3^-, 20; HPO_4^{2-}, 1.6; and glucose, 11. These constituents were dissolved in twice-distilled water just before the start of the experiment. The perfusate was saturated with 95% O_2 and 5% CO_2 at the final temperature and pressure. This provided a pH of 7.42. The apex moves in the direction of the base of the heart at every contraction. The vertical displacement of the apex can be regarded as a parameter of contractile function. The mechanical displacement was converted to an electrical signal by means of a displacement transducer. Displacement was recorded on a Hewlett Packard stripchart recorder (HP 7848A).

After these measurements, a total atrioventricular block was induced at the atrial septum, thus disturbing His bundle conduction.

Fifteen minutes later, after a steady state had been established, the heart was stimulated by a 1-msec square-wave pulse from a current source at twice the threshold value, usually 0.1 mA. The initial pacing frequency was chosen just above the idioventricular rhythm. The stimulation frequency was increased in 25-msec steps for the rat hearts and in 50-msec steps for the rabbit hearts, both of 60 seconds' duration, until no adequate reaction to pacing occurred. Displacement at each frequency was calculated and expressed in percentages of displacement at the lowest frequency. Displacement was plotted against stimulation interval, thus providing a Kruta curve (4). After the experiment, the hearts were perfused with formalin or glutaraldehyde for light- and electron-microscopic examination of the morphologic features.

Gross pathologic examination was performed after each experiment.

RESULTS

During the treatment period, five of the rabbits receiving Adriamycin died of hematologic toxicity and intercurrent infection. Three rabbits were not evaluable because of technical problems during the surgical procedure. Three rats died immediately after the administration of Adriamycin and two died in the tenth week of treatment, probably due to hematologic toxicity.

Pathologic examination resulted in similar findings in both of the animal species studied. The alterations were

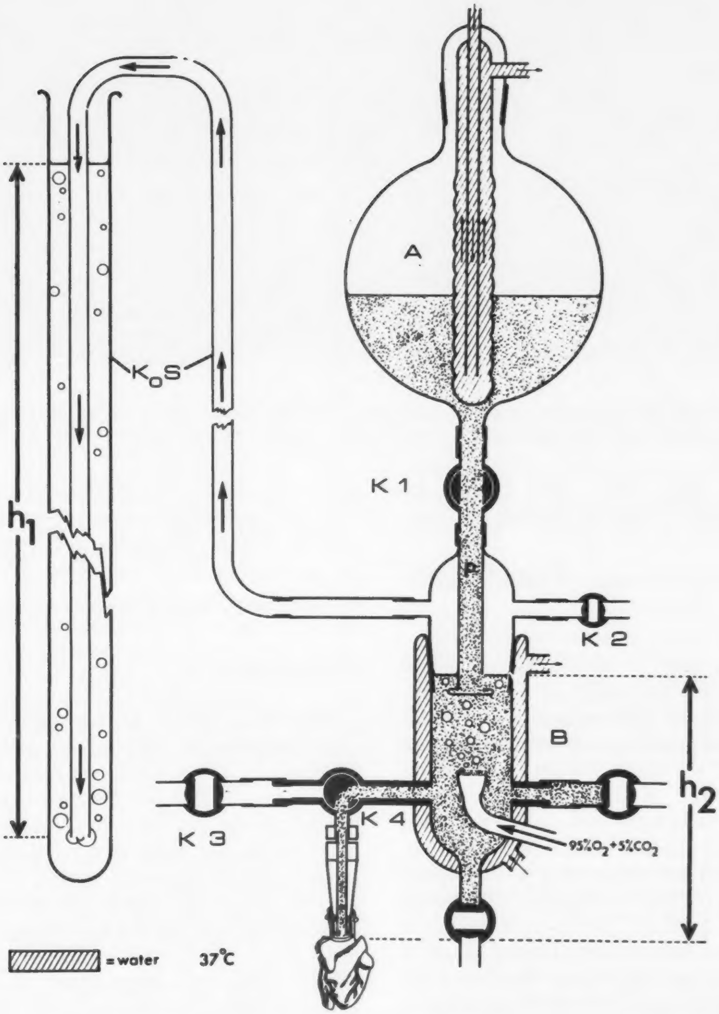

FIGURE 1.—Perfusion apparatus. A: Reservoir in which perfusate is preheated. B: Heating reservoir in which perfusate level can be regulated. P: Hydrostatic pressure. K_1: Stopcock to close the outlet of the reservoir (reservoir can be filled during the experiment). K_2: Accessory stopcock. Opening removes pressure above B. K_3: Stopcock for formalin or glutaraldehyde reservoir. K_4: Stopcock to connect the heart with the perfusate supply. K_o: Water column. S: Rubber tube. h_1: Depth of the tube in the water column. h_2: Difference between the levels in the heating reservoirs and coronary ostia, which determines perfusion pressure.

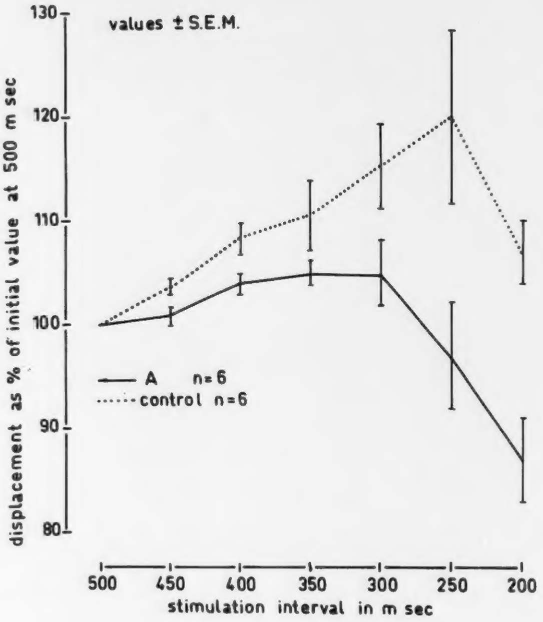

FIGURE 2.—Kruta curves of rabbit hearts. A = hearts exposed to Adriamycin.

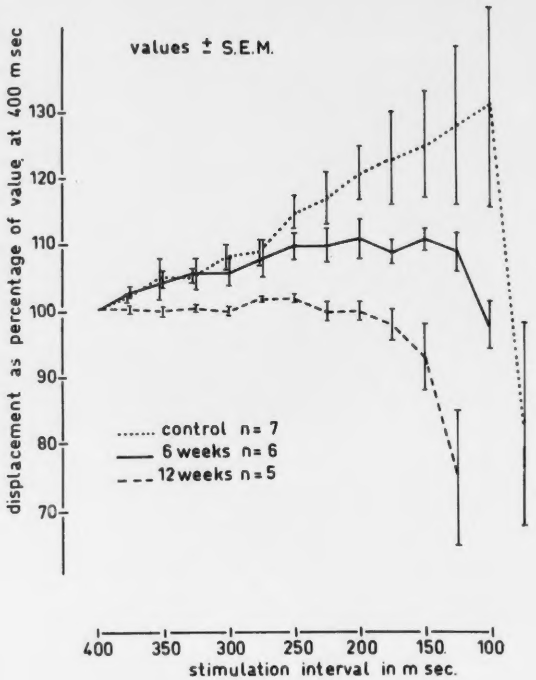

FIGURE 3.—Kruta curves of rat hearts after 6 and 12 wks of exposure to Adriamycin.

characterized by overt signs of cardiac failure: pleural effusions, ascites, nutmeg appearance of the liver, anasarca, and hydropericardium. These alterations were less pronounced in the rats exposed to six doses of Adriamycin than in those given 12 doses. No further histologic investigation was performed. Analysis of the contractile function of the control rabbits and rats showed a gradual increase of displacement with increasing stimulation rate. Maximal apical displacement occurred at a stimulation interval of 250 msecs in the rabbit hearts and at 100 msecs in the rat hearts (figs 2 and 3). The increase was up to 120% of the initial value for the rabbit hearts and up to 135% for the rats. Alternating pulse or fibrillation occurred at stimulation intervals of 200 and 75 msecs in the rabbit and rat respectively. However, the Kruta curve of Adriamycin-treated animals showed signs of impaired contractile function: in the rabbit, maximum displacement was reduced to 103% of the initial value, and the maximum occurred at a longer stimulation interval than in the control rabbits. The isolated rat heart function showed similar abnormalities. Maximal displacement seems to decrease gradually in relation to the dose of the drug. There was no definite stimulation interval at which peak force developed. Alternating pulse or fibrillation occurred at higher stimulation frequencies (fig 4). These factors indicate impaired functional integrity.

DISCUSSION

Adriamycin-induced cardiotoxicity is characterized by electrophysiologic, morphologic, and functional abnormalities which may ultimately result in clinically recognizable and life-threatening congestive heart failure (7). Studies in the rat done by Zbinden and Brändle (8) have shown that there is a relationship between electrocardiographic abnormalities and the total dose administered. In the rabbit, Jaenke found a strong relationship between the dose and the morphologic alterations in the heart tissue (5); histologic examination revealed overt signs of congestive heart failure. These findings have been confirmed by others (9), but the studies did not include electrocardiographic evaluations of cardiotoxicity.

Recently, the rat has been shown to develop morphologic alterations similar to those described in the rabbit (10), and congestive heart failure also occurs in the rat. However, no clear relationship has been found between the electrocardiographic and morphologic alterations (8).

Left ventricular performance may be considered the sum of preload or muscle fibril length, afterload or impedance to the injection of the blood, and the contractile state of the myocardium, which refers to the intrinsic ability of the heart to generate force independent of such factors

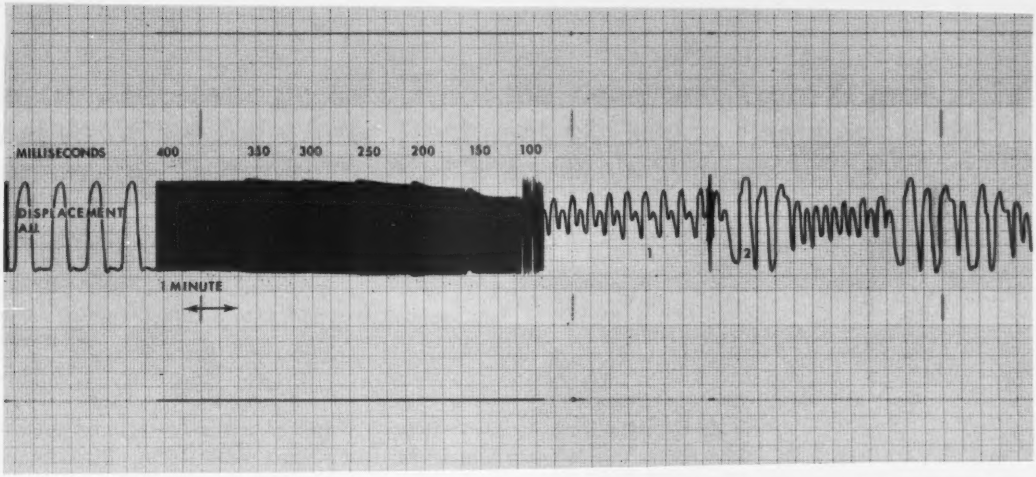

FIGURE 4.—Displacement curve (arbitrary units) of a rat heart after 6 wks of exposure to Adriamycin at a stimulation interval of 100 msecs. An adequate reaction to stimulation did not occur, which results in an alternating pulse (1) or fibrillation (2).

as low-loading and catecholamines. Assessment of the functional integrity of the heart after in vivo exposure to drugs, ie, assessment of contractility under standardized conditions of preload and afterload, and with the use of a standardized perfusion medium, provides a valuable and reliable parameter with predictive value for the cardiotoxic potency of anthracyclines. Interval-force curves have been shown to reflect this contractile function accurately (11). Although a variety of methods for the assessment of contractility have been described (12), the present method deserves preference because of the relatively simple procedure. The rat has proved to be an almost ideal animal model for cardiotoxicity studies. Moreover, this species has recently been shown to offer a particularly useful system for the monitoring of objective tumor response as well as of development of cardiotoxicity by new anthracycline analogs (13). This combination of qualities has not been described in the rabbit. The results of the present study suggest that the decline of contractile properties in the hearts of both species is dose-related. These findings are consistent with the dose-related electrocardiographic and morphologic alterations described for the rat and the dose-related morphologic alterations described for the rabbit. Our findings suggest that the decline in contractility is progressive. In the search for analogs with equal oncolytic action and fewer cardiotoxic side effects, characterization of contractile decline is of paramount importance.

Evaluation of the functional integrity of isolated perfused hearts on the basis of Kruta curves provides a unique possibility to combine electrophysiologic, morphologic, and functional criteria for the screening of anthracyclines with respect to cardiotoxicity.

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Electron Microscopic Studies of the Heart and Light Microscopic Studies of the Skin After Treatment of Golden Hamsters With Adriamycin, Detorubicin, AD-32, and Aclacinomycin[1]

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With the technical assistance of I. Pignot, M. Bennoun, and G. Martin[2]

SUMMARY

Golden hamsters received Adriamycin (ADR), detorubicin (DTR), AD-32, or aclacinomycin (ACM) three times weekly at doses extrapolated from optimal doses in L1210 leukemia. Minimal early lesions of the myocardium were detected by electron microscopy in ACM-treated animals. These lesions were aggravated after several weeks of treatment but remained reversible. Lesions in AD-32-treated hamsters were slightly more marked than those seen in ACM-treated animals but were much less severe than those observed in ADR- or DTR-treated animals. Similarly, light microscopy revealed pathologic changes in the skin following ADR or DTR administration. These changes were not seen in animals receiving AD-32 or ACM.

[Cancer Treat Rep 63:875–888, 1979]

Two important limiting factors for the use of Adriamycin (ADR) are cardiotoxicity and alopecia. Many anthracycline derivatives have been prepared in the hope that some would show reduced toxic effects. In phase II trials with detorubicin (DTR) (1) and aclacinomycin (ACM) (2) we have observed that DTR produces cardiotoxicity (30%) and alopecia (90%) as frequently as ADR, whereas ECG changes were rare (4.5%) and no alopecia was encountered with ACM. A lower incidence of alopecia has also been reported with AD-32 (3).

Myocardial lesions have been described by others in electron microscopic (EM) studies in patients treated with daunorubicin (4) and ADR (5). The present work was designed to determine if EM findings in the myocardium of Golden hamsters receiving anthracyclines could be predictive of the cardiotoxic potential of these agents in man. We have also examined the skin of these animals by light microscopy in an attempt to define a correlation between histologic changes in these experiments and the development of alopecia in man. Our investigations were performed with ADR, DTR, AD-32, and ACM.

MATERIALS AND METHODS

Twenty-one Golden hamsters were used for each compound and 21 were kept as controls. Treatments were given ip three times a week for 1–4 weeks. The dose per injection for each compound was three fourths of the optimal dose per injection in L1210 leukemia on a qd, Days 1, 5, and 9 schedule (6).

Survival and weight were routinely followed. Three hamsters per treatment group and three per control group were killed each week. All tissues of interest were quickly removed and the ventricular cross (apices) was immediately fixed for transmission EM study in 5% cacodylate buffered glutaraldehyde adjusted to pH 7.25; the apex of the myocardium was minced under fixative into 1–2-mm^3 blocks.

The remainder of the heart and other tissues (including lymph nodes, thymus, spleen, liver, lungs, kidneys, intestine, bones, ovaries, muscles, and skin) were collected

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for light microscopic examination and fixed in 10% neutral buffered formalin.

The blocks for transmission EM studies were postfixed in 2% osmium tetroxide, dehydrated in graded ethanols, transferred to propylene oxide, and embedded in Epon. Thin sections cut at 300–500 Å with a Porter-Bloom ultramicrotome were mounted on Formvar-coated 300-mesh copper grids, stained with uranyl acetate and lead citrate, and examined with a Jeol 100 C ASSID electron microscope at 100 kv.

For histopathologic study the tissues were embedded in paraffin, sectioned at 6 μm, and stained with hematoxylin and eosin. At the present time only the heart and the skin have been examined by light microscopy.

Our grading system for EM alterations was derived from that proposed by Billingham et al (5). Grade 0 was used for a normal cardiac morphology and grade 1 for the cases which contained some cardiac cells with mild alterations, ie, swelling of mitochondria with clearing of their matrices and lysis of their crests, and lesions of myofilaments in some cells presenting a separation, a loss of their parallel orientation, and a lysis with disruption of Z-band registry. Grade 2 was assigned when some cardiac cells demonstrated the previous alterations, but more marked; these included vacuolization of mitochondria, appearance of myelinic figures, dilatation of sarcoplasmic reticulum, more intense loss of myofilaments, nuclear modifications with irregularity of the nuclear membrane and condensation or clumping of the chromatin into electron-dense masses adjacent to the nuclear membranes, and separation of intercalated discs. In grade 3, the myocardium had diffuse, severe alterations, with complete cell disorganization, vacuolization, loss of contractile elements, and near cell death.

To establish a possible comparative appreciation of the myocardial alterations, we also used the intermediate grades of 0–1, 1–2, and 2–3, which indicate that about half of the myocardial cells showed the morphologic structure or changes of each of the two grades, eg, grade 0–1 signifies that half of the observed cells were normal (grade 0) and that half had grade 1 lesions.

RESULTS

Mortality

Mortality was higher with ADR and DTR than with AD-32 and ACM (table 1). Before death, 35% ± 7% and 30% ± 5% loss of initial weight was observed in ADR- and DTR-treated animals respectively, which suggested that death was probably not due, or not entirely due, to cardiac failure. There was no loss of weight in AD-32- and ACM-treated animals. Loss of hair was evident in ADR and DTR groups but did not appear in the other treatment groups.

TABLE 1.—Mortality of drug-treated animals

	No. of animals surviving at end of—			
Drug*	1st wk	2nd wk	3rd wk	4th wk
ADR	21	13	1	0
DTR	21	14	5	0
AD-32	21	18	15	11
ACM	21	18	14	11
Controls	21	18	15	12

*ADR, DTR, AD-32, and ACM were given 3 times a wk for 1–4 wks at 3, 3, 30, and 6 mg/kg/injection respectively. 3 animals were killed each week. If there were < 3 survivors, all were killed.

Cardiac Lesions Shown by Electron Microscopic Studies

Generally, the main types of cardiac ultrastructural alterations observed in our study of the cardiotoxicity of anthracyclines concerned myofibrils, Z-bands, mitochondria, sarcoplasmic reticulum, nucleus, and intercalated discs. The myofibrils showed tears and lysis of myofilaments, with disappearance of Z-bands. The alterations in the mitochondria were characterized by swelling, clearing of their matrices, disappearance of their crests, and formation of vacuoles and myelinic figures. The dilatation of sarcoplasmic reticulum led to large, membrane-bound clear spaces or vacuolizations in the cytoplasm. The condensation of the nuclear chromatin formed electron-dense masses, most of which were adjacent to the nuclear membrane. Finally, the separations of the fascia adherens of the intercalated discs created empty spaces between myocytes. If the treatment with anthracyclines continued for > 1 week, the alterations became progressively more severe. The myocytes lost their myofibrils and mitochondria, and finally became filled with vacuoles of different sizes.

The observed modifications were semiquantified according to the criteria defined above (table 2).

End of the First Week (After Three Drug Injections)

With ADR, the myocardium of the three animals that were killed showed very important ultrastructural alter-

TABLE 2.—Degree of myocardial alterations at end of each wk

	Degree† of myocardial alterations at end of—			
Drug*	1st wk	2nd wk	3rd wk	4th wk
ADR	1–2	2–3	2–3	—
DTR	1	1–2, 2	2–3	—
AD-32	1	1–2	2	2, 2–3
ACM	0–1	0–1, 1	1–2	2, 2–3
Controls	0	0	0	0

**See* footnote to table 1 for doses.

†0 = no pathologic cell alterations; 1 = moderate cell alterations; 2 = severe cell alterations; 3 = very severe alterations just before cell death (*see* text for description of precise microscopic findings).

ations: (*a*) swelling of mitochondria with clearing of their matrices, disappearance of their crests, and formation of vacuoles and myelinic figures; (*b*) tears and loss of myofilaments and Z-bands; (*c*) widening of sarcoplasmic reticulum with vacuolization of the cytoplasm; (*d*) separation of the external membrane from the internal membrane of the nucleus; and (*e*) separation of intercalated discs. Figure 1 shows a typical example of these alterations, which may be classified as grade 1–2 according to the semiquantitative grading system described previously.

With DTR, the lesions were not different from those described for ADR but were seen less often. Myocytes showed swelling of mitochondria with clearing of their matrices, dilatation of sarcoplasmic reticulum, and lysis of myofilaments with disruption of Z-bands. Figure 2 shows a characteristic type of these alterations, which are classified as grade 1.

With AD-32, the lesions were also less marked than after treatment with ADR. They consisted of swelling of mitochondria, clearing of their matrices, dilatation of sarcoplasmic reticulum, and separation and lysis of myofilaments with disruption of Z-bands. Figure 3 shows a typical example of these lesions, classified also as grade 1.

With ACM, the lesions were less frequent and much less severe. Generally, mitochondria, myofilaments, and intercalated discs were well-preserved. However, it was possible to find dilatation of the sarcoplasmic reticulum. Figure 4 is representative of this finding which is classified as grade 0–1.

End of the Second Week (After Six Drug Injections)

The myocardium of ADR-treated animals showed an increase in all alterations already seen at the end of the first week. The disappearance of myofibrils, the vacuolization of sarcoplasm, the condensation of nuclear chromatin, and the total disorganization of the myocytes were classified as grade 2–3 changes (fig 5).

The lesions of DTR-treated animals were also very marked but less intensive than those which appeared soon after ADR treatment, ie, swelling of mitochondria with clearing of their matrices and lysis of their crests, separation, disorganization, and lysis of myofilaments with disruption of Z-band registry, formation of myelinic figures, and separation of the fascia adherens of the intercalated discs (fig 6). These cases were classified as grades 1–2 and 2.

The lesions of AD-32-treated animals were significant but less intensive than those seen with ADR. The findings consisted of irregular nuclear membranes and condensation of the chromatin into electron-dense masses, which were adjacent to the nuclear membranes and to the nucleoli (fig 7); these cases were classified as grade 1–2.

In ACM-treated animals, the lesions began to appear but were still very mild. Most frequently, the myofilaments and mitochondria were well-preserved. Some myocytes showed swelling of mitochondria with clearing of their matrices and lysis of crests. Contractions of sarcomeres were also seen (fig 8). These cases were classified as grades 0–1 and 1.

End of the Third Week (After Nine Drug Injections)

There was only one surviving hamster in the group of ADR-treated animals and there were only five in the group of DTR-treated animals. These animals presented the same severe myocardial signs of degeneration as those seen at the end of the second week. However, some myocytes were only moderately altered and moreover some were relatively well-preserved. These cases were classified as grade 2–3.

The myocardia of AD-32-treated animals (even if no hamster was dead at that time) also showed severe alterations of mitochondria, myofilaments, and intercalated discs. These lesions were classified as grade 2.

At the same time the myocardia of ACM-treated animals still showed only moderate changes which were classified as grade 1–2.

End of the Fourth Week (After 12 Drug Injections)

The only surviving hamsters were found in the groups of AD-32- and ACM-treated animals. The myocardia of the killed animals in these two groups contained almost all types of previously described alterations, and some cells showed the same degree of cell degeneration as those of the ADR- and DTR-treated animals at the end of the third week. However, there was a clear difference between the well-preserved general condition of these ACM- and AD-32-treated animals at the end of the fourth week, without loss of weight and the very sick appearance of ADR- and DTR-treated animals.

The ACM and AD-32 myocardial lesions seen at the end of the fourth week were classified as grade 2 to 2–3 as were those observed after ADR and DTR treatment at the end of the third week.

Recovery of Electron-Microscopic Myocardial Alterations in Surviving ACM- and AD-32-Treated Animals 2 Months After Interruption of Treatment

Two months after the end of ACM and AD-32 treatment (12 drug injections in 4 weeks), three animals from each group were killed and their myocardia were submitted to EM study. Myofibrils and their mitochondria were generally as well-preserved in ACM- and AD-32-treated animals as they were in the controls. The rare EM abnormalities found consisted of moderate vacuolization of the cytoplasm and some separations of the intercalated discs (fig 9). The morphologic aspects of the large number of myocytes were identical to those of controls.

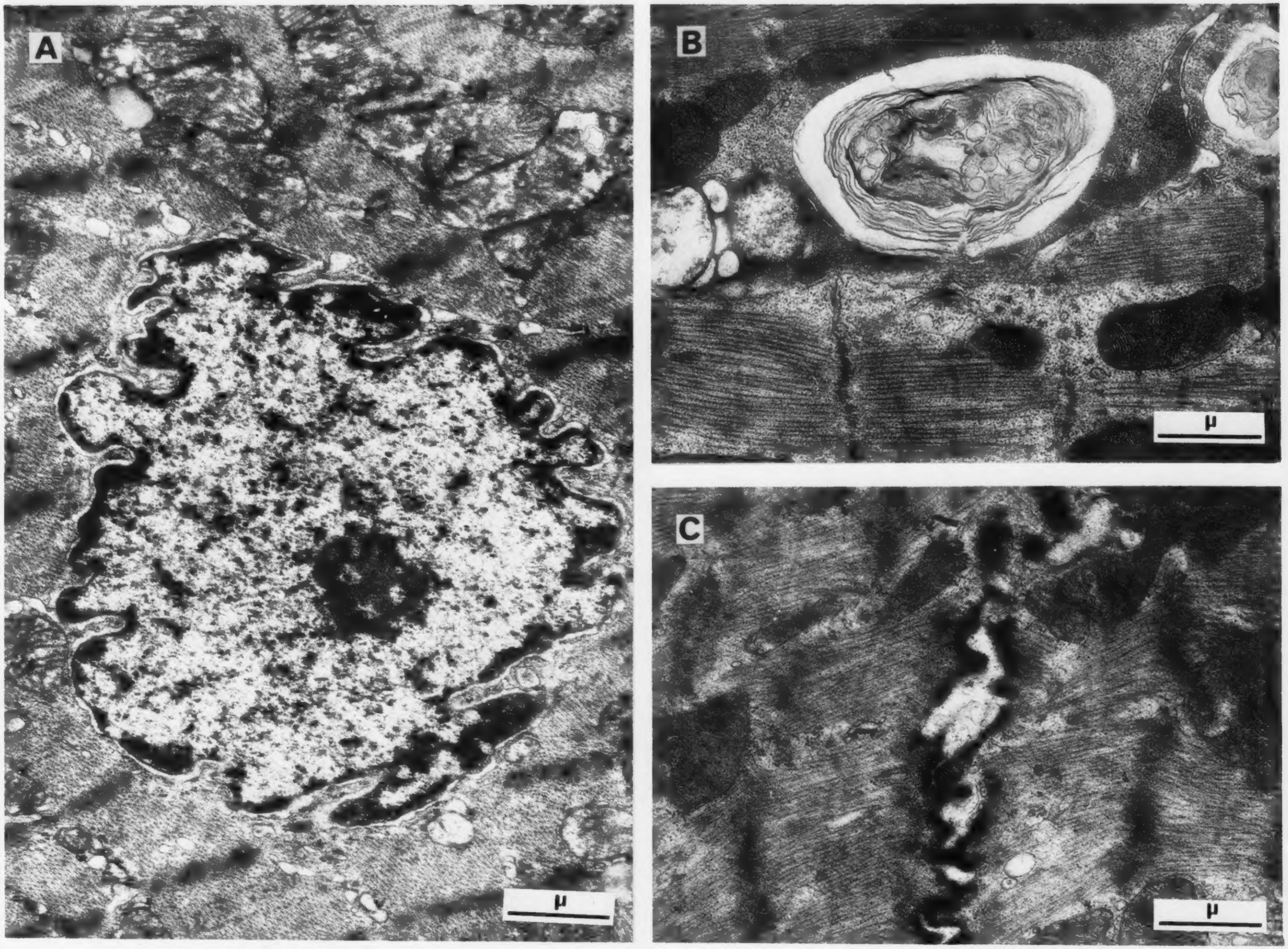

FIGURE 1.—Myocardium from a Golden hamster 7 days after treatment with ADR (3 mg/kg/injection ip 3 times a wk). Swelling of mitochondria with clearing of their matrices, disappearance of their crests (A), and formation of vacuoles and myelinic figures (B). Tears and loss of myofilaments and widening of sarcoplasmic reticulum (B). Irregular nuclear membrane, condensation of the chromatin into electron-dense masses adjacent to the nuclear membrane, and separation of external membrane from internal membrane of the nucleus (A). Separation of fascia adherens of the intercalated discs (C). Original magnification (6/9): A, × 8300; B, × 8300; C × 8300.

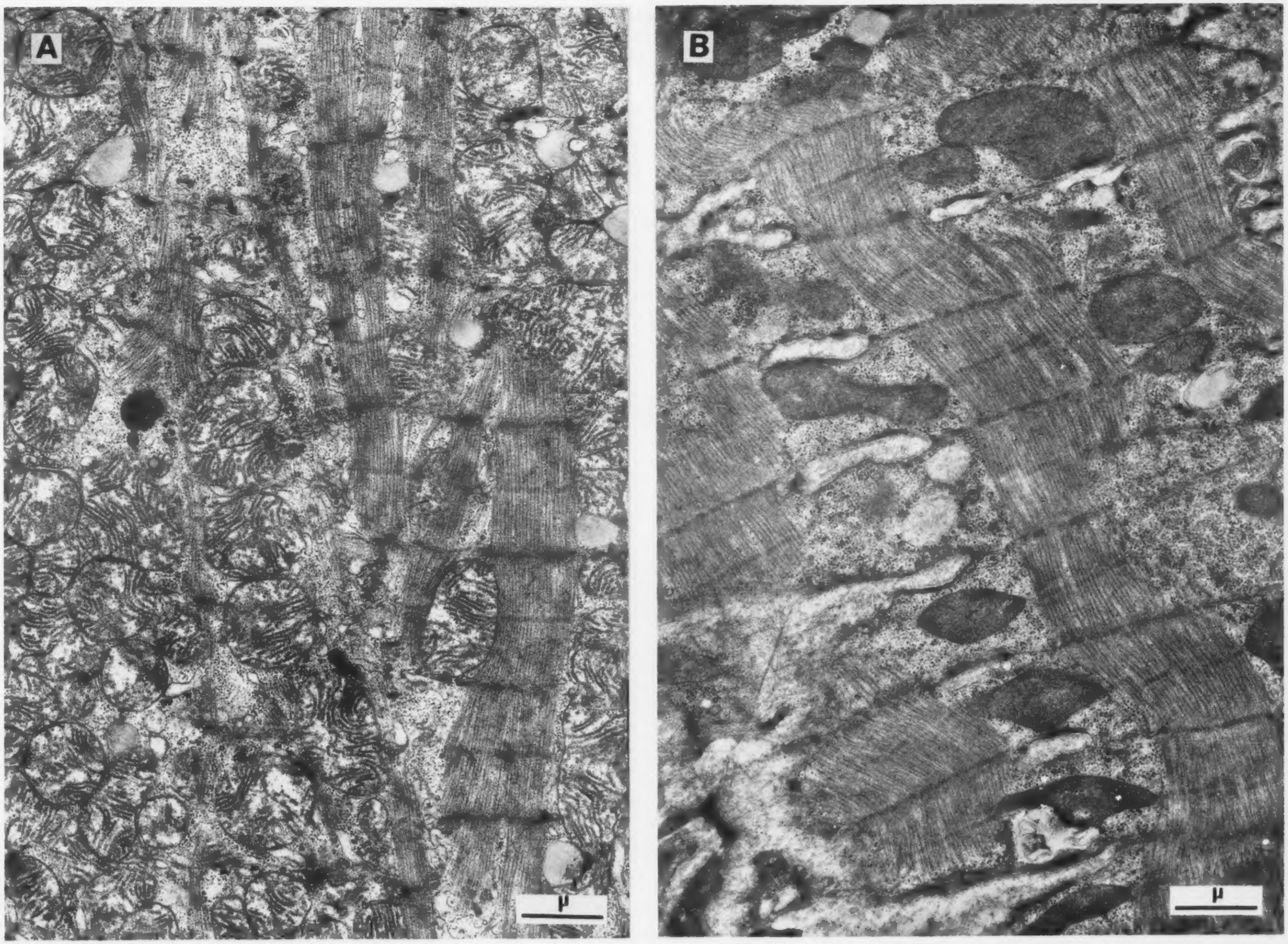

FIGURE 2.—Myocardium from a Golden hamster 7 days after treatment with DTR (3 mg/kg/injection ip 3 times a wk). Swelling of mitochondria with clearing of their matrices and lysis of the crests (A). Dilatation of sarcoplasmic reticulum (B). Separation and lysis of myofilaments with disruption of Z-band registry (A and B). Original magnification (6/9): A, × 6600; B, × 6600.

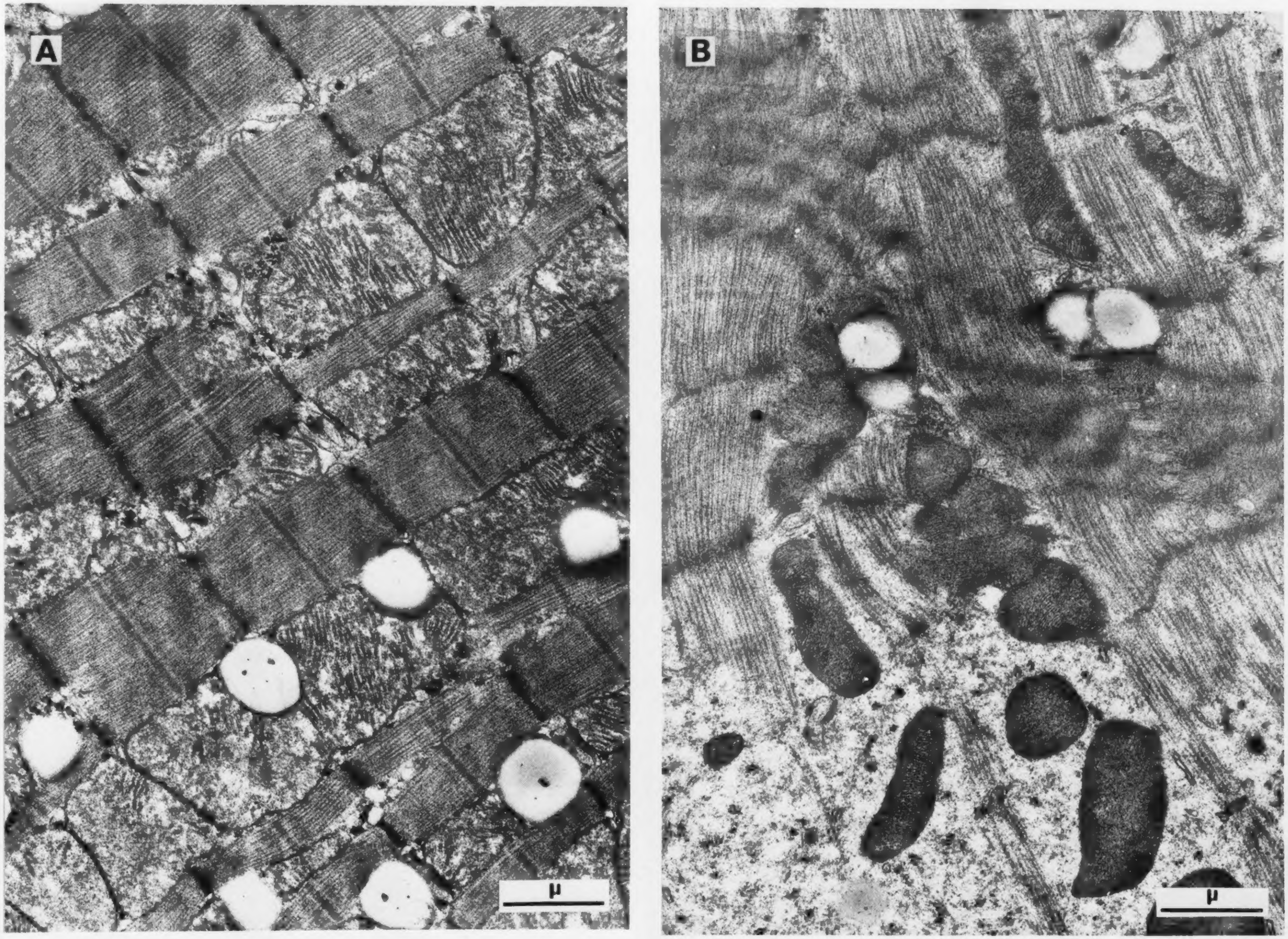

FIGURE 3.—Myocardium from a Golden hamster 7 days after treatment with AD-32 (30 mg/kg/injection ip 3 times a wk). Swelling of mitochondria with clearing of their matrices and lysis of their crests (A). Dilatation of sarcoplasmic reticulum (A and B). Separation and lysis of myofilaments with disruption of Z-band registry and contraction of sarcomeres (B). Original magnification (6/9): A, × 8300; B, × 8300.

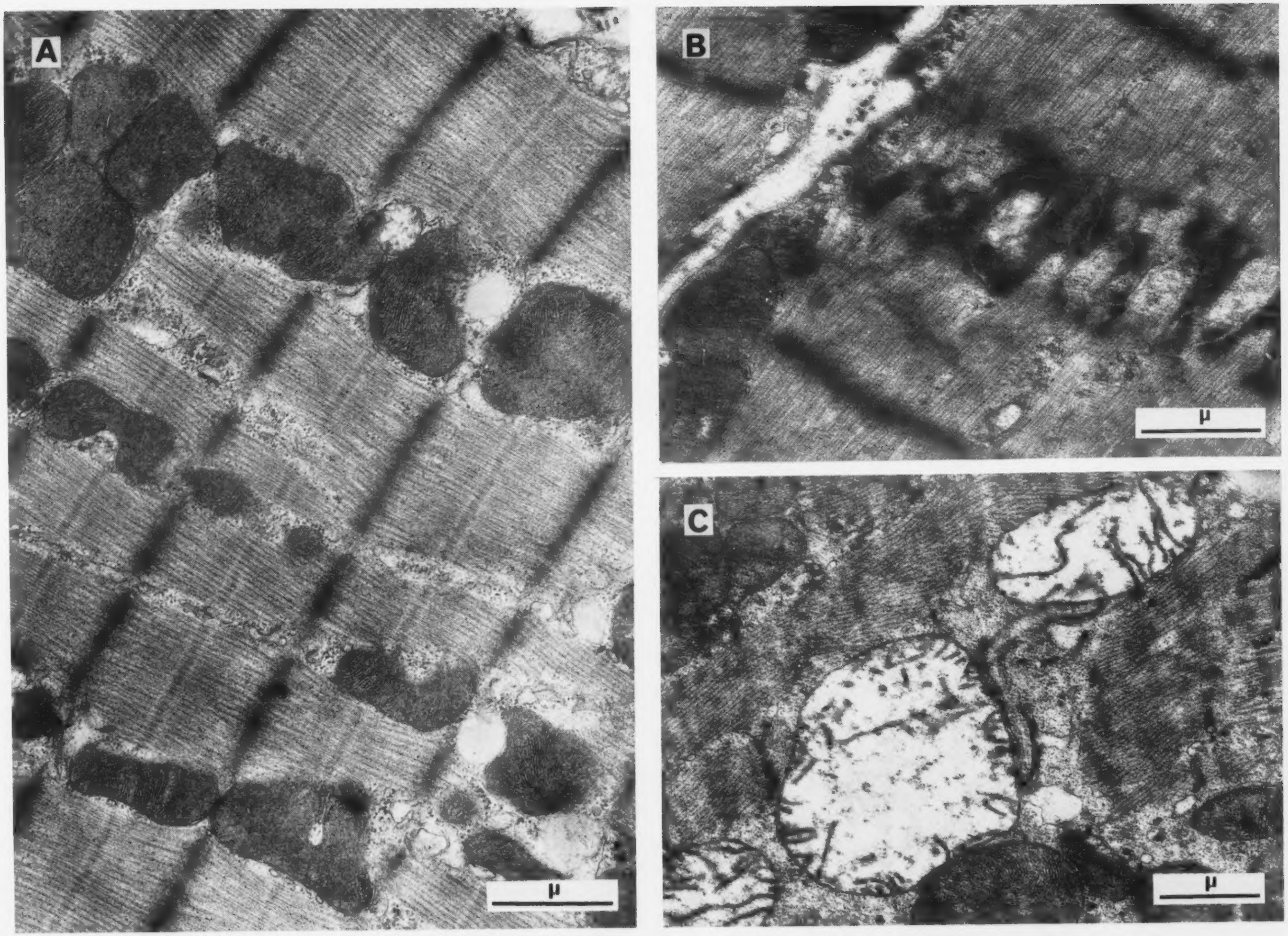

FIGURE 4.—Myocardium from a Golden hamster 7 days after treatment with ACM (6 mg/kg/injection ip 3 times a wk). Generally, mitochondria, myofilaments, and intercalated discs are well-preserved (A and B), but it is possible to find clearing of the matrices of some mitochondria and moderate dilatation of the sarcoplasmic reticulum (C). Original magnification (6/9): A, × 10,000; B, × 10,000; C, × 8300.

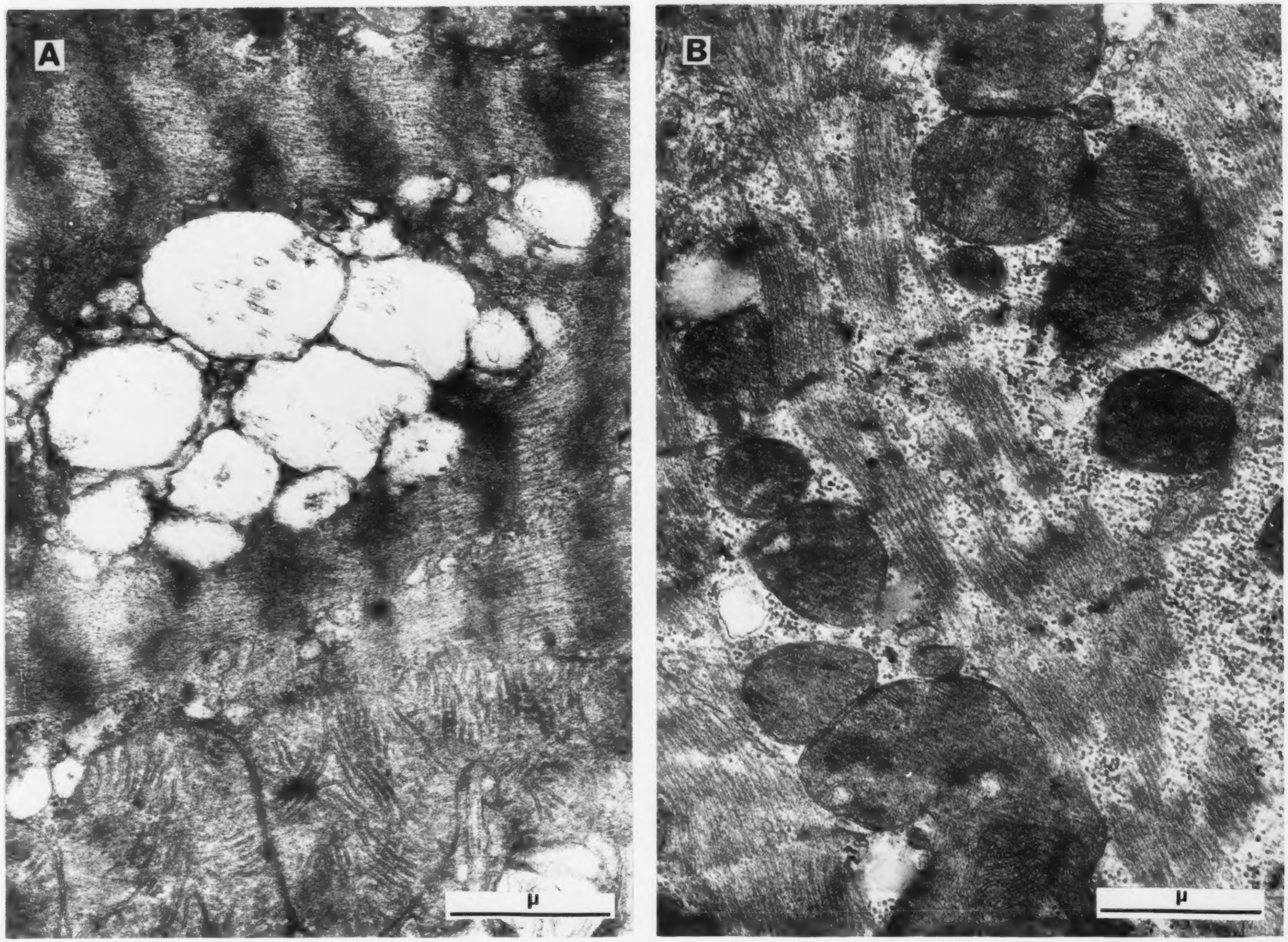

FIGURE 5.—Myocardium from a Golden hamster 14 days after treatment with ADR (3 mg/kg/injection ip 3 times a wk). Swelling of mitochondria with clearing of their matrices, lysis of their crests, vacuolization, and formation of myelinic figures (A). Separation, loss of parallel orientation, and lysis of myofilaments with disruption of Z-band registry (B). Contraction of sarcomeres (A). Original magnification (6/9): A, × 1300; B, × 1300.

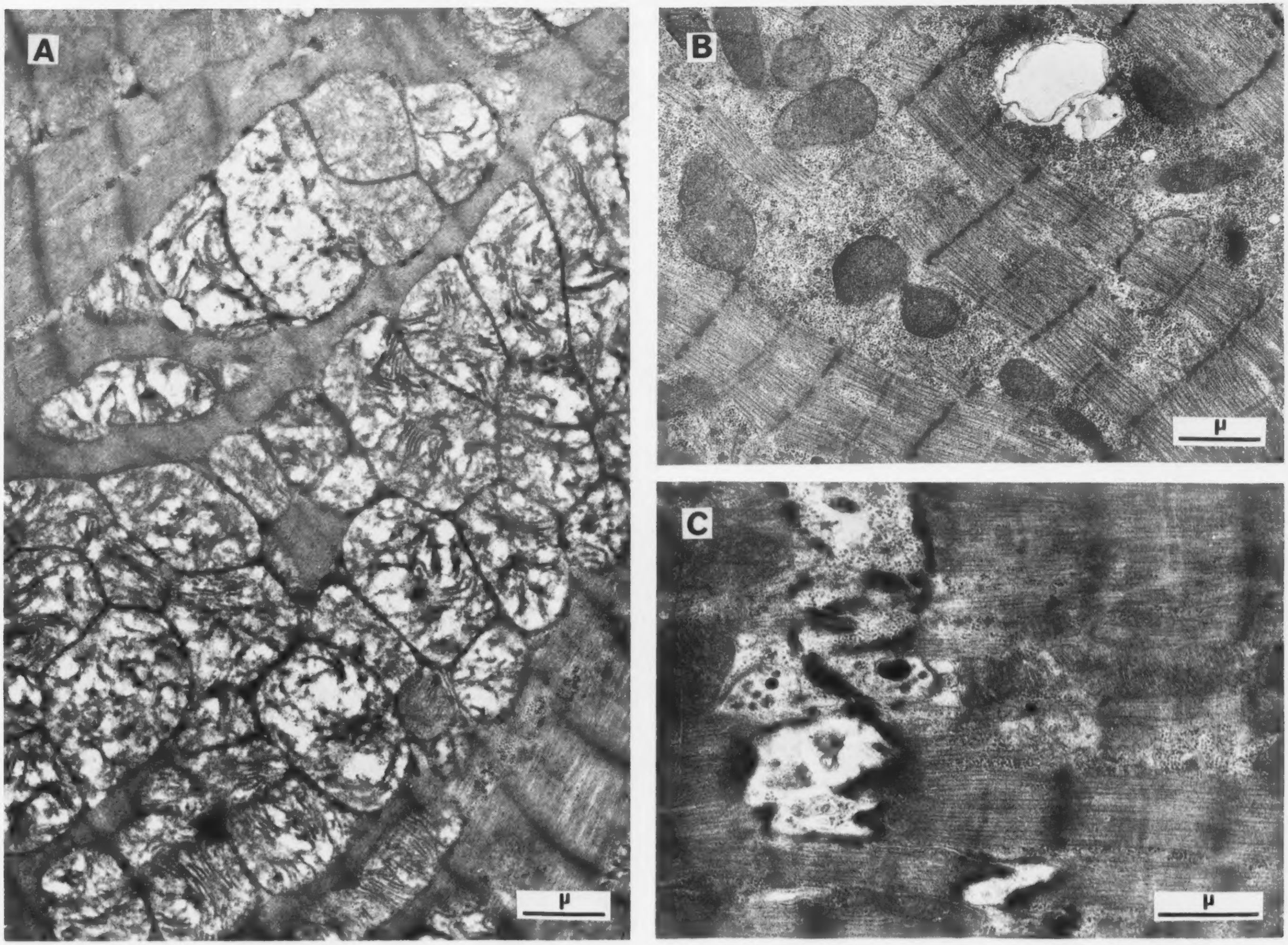

FIGURE 6.—Myocardium from a Golden hamster 14 days after treatment with DTR (3 mg/kg/injection ip 3 times a wk). Swelling of mitochondria with clearing of their matrices and lysis of their crests (A). Separation, disorganization, and lysis of myofilaments with disruption of Z-band registry (B). Dilatation of sarcoplasmic reticulum and formation of vacuoles (B). Separation of fascia adherens of the intercalated discs (C). Original magnification (6/9): A, × 6600; B × 6600; C × 8300.

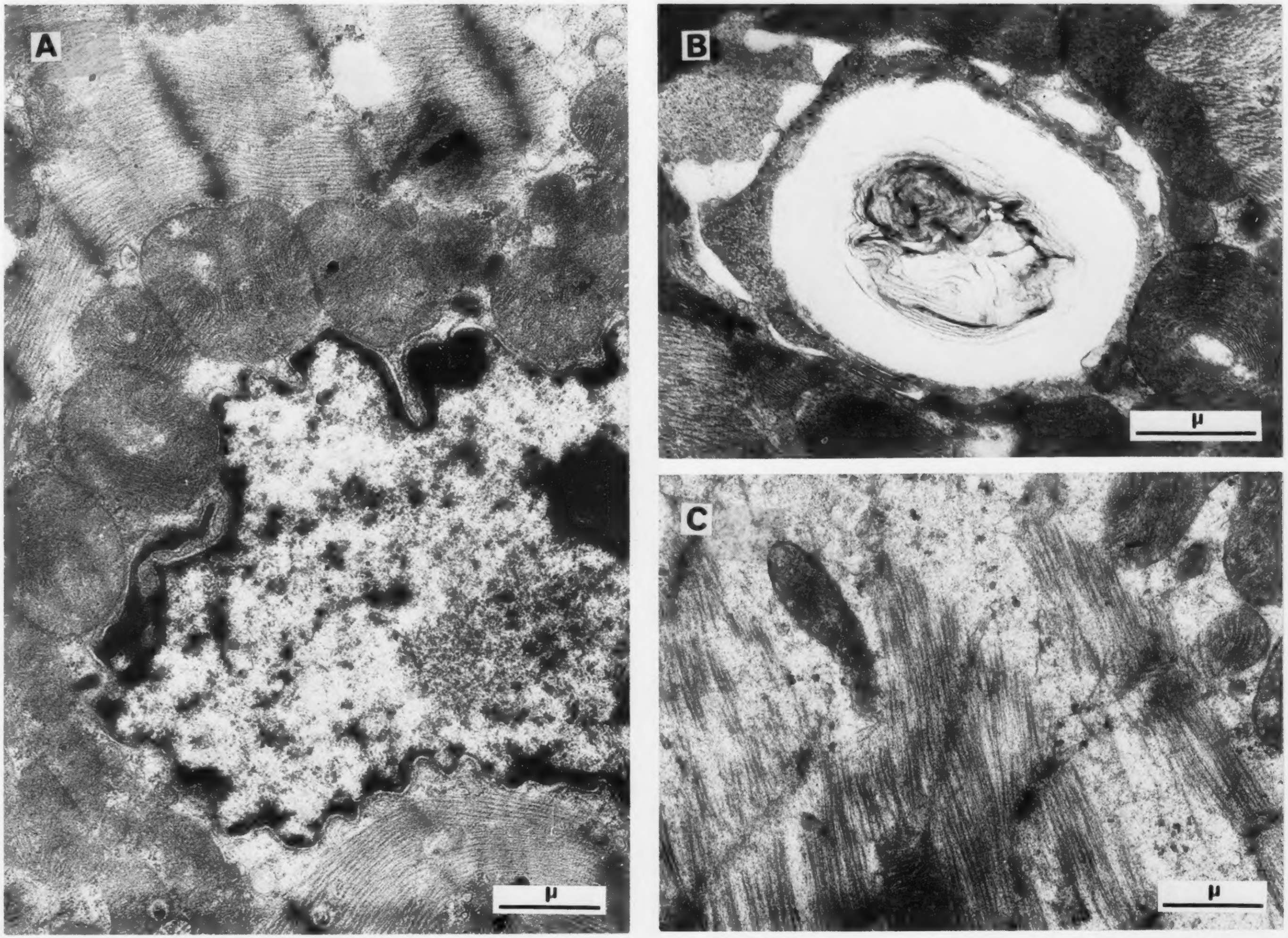

FIGURE 7.—Myocardium from a Golden hamster 14 days after treatment with AD-32 (30 mg/kg/injection ip 3 times a wk). Irregular nuclear membranes and condensation of the chromatin into electron-dense masses which are adjacent to nuclear membranes (A). Dilatation of sarcoplasmic reticulum and formation of myelinic figures (B). Separation and lysis of myofilaments with disruption of Z-band registry (C). Original magnification (6/9): A, × 8300; B, × 10,000; C, × 8300.

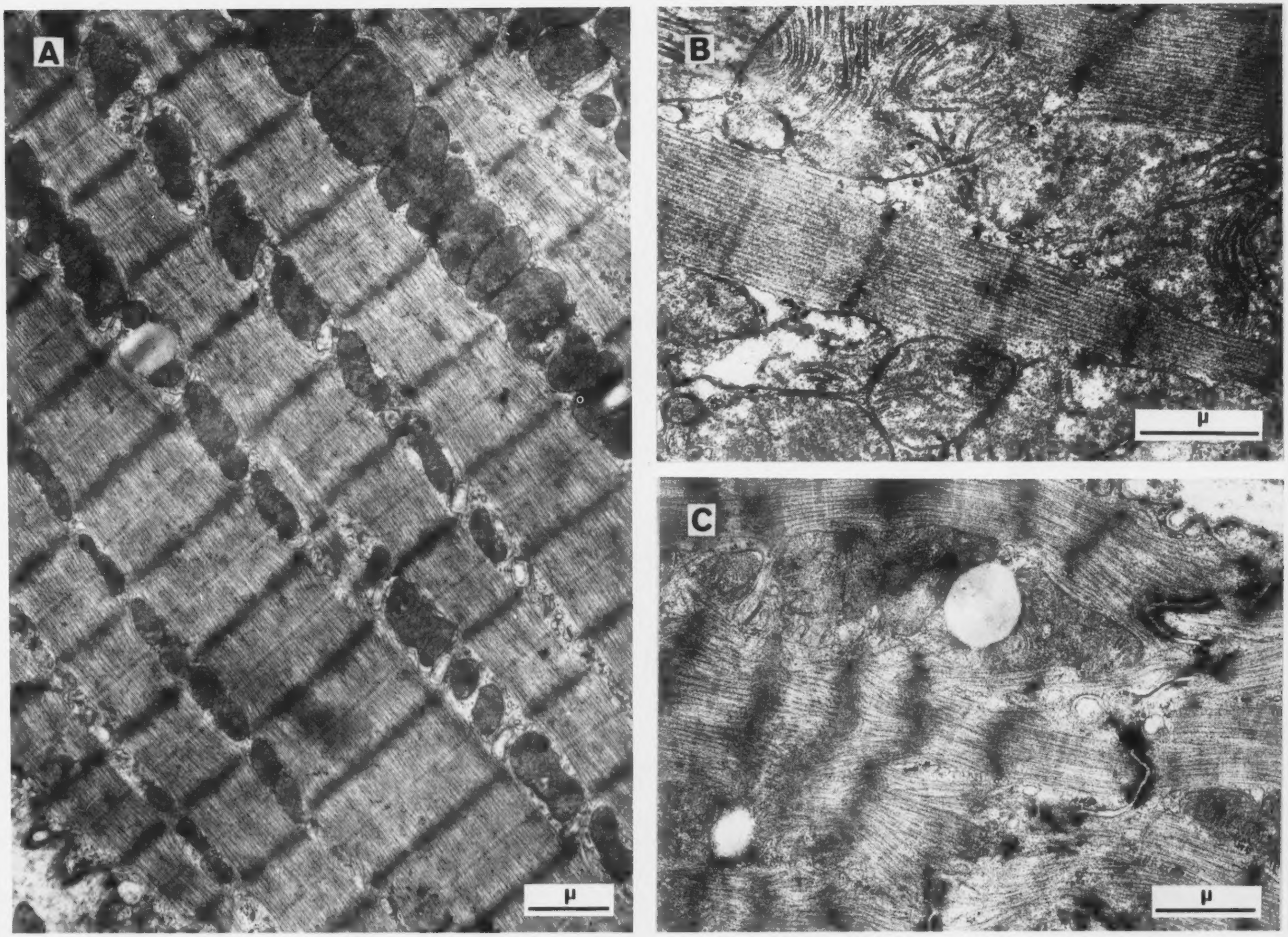

FIGURE 8.—Myocardium from a Golden hamster 14 days after treatment with ACM (6 mg/kg/injection ip 3 times a wk). Most frequently, myofilaments and mitochondria are well-preserved (A) but some myocytes show swelling of mitochondria with clearing of their matrices and lysis of their crests, dilatation of sarcoplasmic reticulum (B), and contraction of sarcomeres (C). Original magnification (6/9): A, × 6600; B, × 10,000; C × 8300.

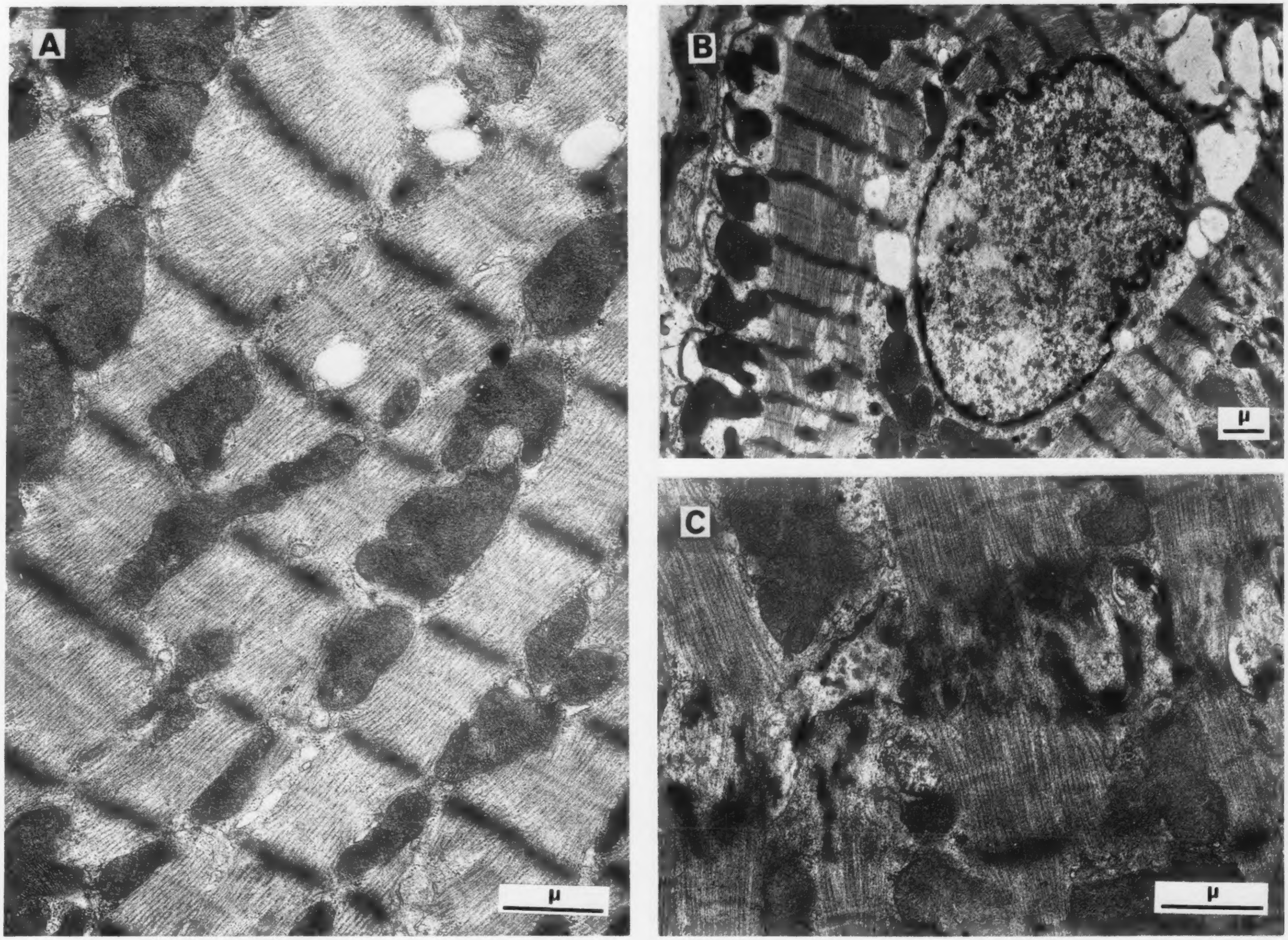

FIGURE 9.—Myocardium from a Golden hamster 8 wks after administration of 12 injections of AD-32 or ACM (treatment given over 4 wks for each anthracycline). Generally, myofilaments and mitochondria are in good condition (A and B) but some alterations persist, such as moderate vacuolization of the cytoplasm (B) and some separation of the intercalated discs (C). Original magnification (6/9): A, × 8300; B, × 3300; C, × 8300.

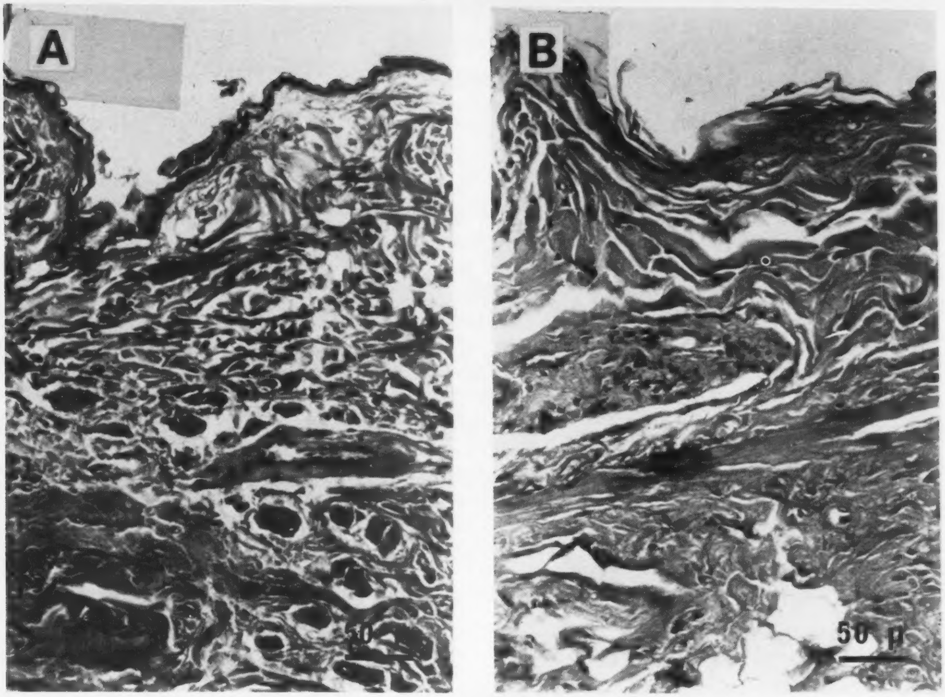

FIGURE 10.—Histopathologic alterations of the skin of Golden hamsters after treatment for 3 wks with ADR (A) and DTR (B). Atrophy of all layers of the epidermis and loss of hair occurred. Original magnification (24/36): A, × 60; B, × 60.

Skin Changes Shown by Light Microscopic Studies

In the groups of ADR- and DTR-treated animals, the histopathologic study of the skin showed degenerative lesions which progressed every week. Although it was difficult to distinguish typical lesions at the end of the first week, after the second week of ADR or DTR treatment, the skin showed very marked atrophy of all layers of epidermis and a loss of hair as shown in figure 10. On the contrary, the skin of AD-32- and ACM-treated animals preserved its normal histologic structure even after 4 weeks of treatment (fig 11).

DISCUSSION

Our findings seem to indicate a lesser degree of cardiotoxicity and skin toxicity with AD-32 and ACM as compared to ADR and DTR. These results are very consistent with clinical data presently available with these compounds (1–3, 7, 8). However, the clinical information on these ADR derivatives is still preliminary and our experimental observations are tempered by several considerations.

Since anticancer agents are administered in man at doses close to the maximum tolerated dose, comparison for specific toxic effects (eg, cardiotoxicity) between analogs must ideally be undertaken either at the same therapeutic index or relative to other toxic effects (eg, myelosuppression). The former approach has been selected for our investigations. However, extrapolating dose schedules for Golden hamsters from optimal doses at a particular schedule in L1210 murine leukemia proved to be somewhat inaccurate. This procedure resulted in the administration of doses that obviously led to greater general toxicity in animals treated with ADR and DTR as compared to that encountered with the other treatments.

The relative degree of all toxic effects induced by the anthracyclines investigated in this study cannot yet be properly ascertained. This question will hopefully be clearly answered by a careful light microscopic analysis of all organ samples collected from killed animals. This analysis is ongoing but it would be premature to draw any conclusion at this point.

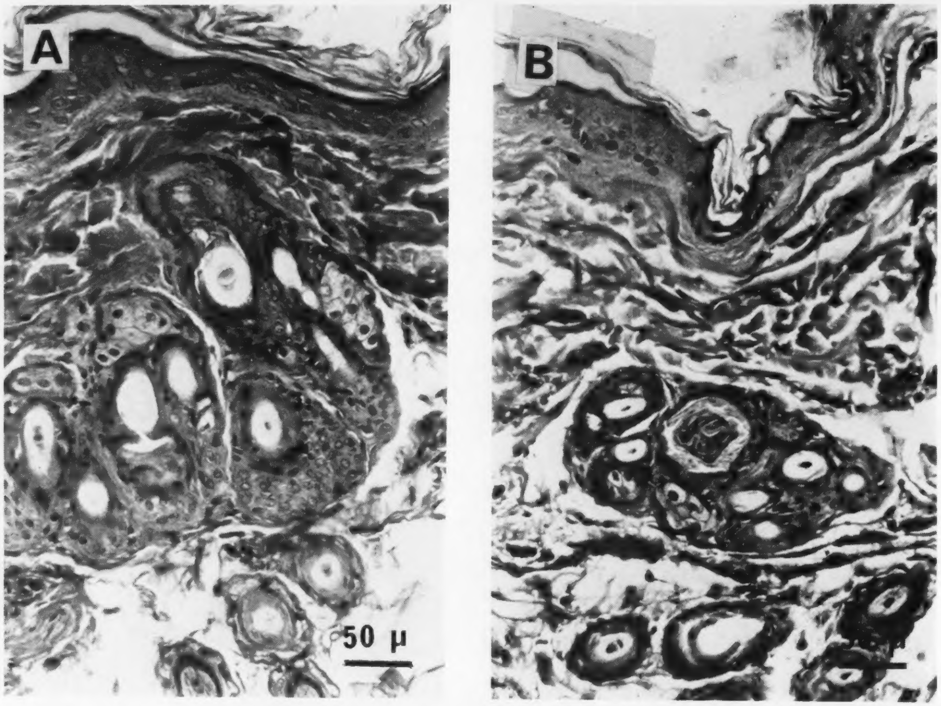

FIGURE 11.—Histopathologic alterations of the skin of Golden hamsters after treatment for 4 wks with ACM (A) and AD-32 (B). Normal histologic structure of epidermal cell layers and no alopecia occurred. Original magnification (24/36): **A**, × 60; **B**, × 60.

Cardiac lesions were seen with all anthracyclines included in this analysis. Most interesting was the demonstration of a possible recovery of cardiac lesions. This occurred relatively rapidly following moderate cardiac damage produced by AD-32 and ACM but might conceivably also occur with other cardiotoxic anthracyclines. The practical impact of these findings remains to be established in man.

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Clinical Activity of Detorubicin: A New Anthracycline Derivative[1]

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SUMMARY

The anthracycline derivatives are intercalating drugs which are of major importance in the treatment of leukemias and in the management of solid tumors. Structural analogs have been prepared by semisynthetic modifications in an attempt to extend the spectrum of antitumor activity and to reduce toxicity (acute myelosuppression and cardiotoxicity). This report concerns our preliminary clinical experience in 111 patients who received detorubicin. Two dose schedules were used in acute leukemia patients. Sequential doses were active in acute leukemia relapses but the mucous membrane toxicity was excessive; more recently, intermittent doses proved active in acute leukemia relapses (one 6-mg/kg dose) and in a patient with resistant Burkitt's lymphoma. In non-Hodgkin's lymphomas, a complete response rate of 71% was achieved with an intermittent schedule (3 mg/kg/day × 3 weeks). A remarkable shrinkage of skin involvement was also observed. Detorubicin showed a high activity in mycosis fungoides (five regressions among six patients) and some activity in soft tissue sarcomas, osteosarcomas, and various solid tumors.

[Cancer Treat Rep 63:889–893,1979]

The anthracycline derivatives are intercalating drugs which are used in the treatment of leukemias and in the management of solid tumors. However, two toxic effects limit the dose of these compounds: acute myelosuppression and congestive heart failure (cumulative dose-dependent delayed cardiomyopathy). Many structural analogs have been prepared by semisynthetic modifications in an attempt to extend the spectrum of antitumor activity and to reduce toxicity (1). Among them, detorubicin (DTR) (14-diethoxacetoxy-daunorubicin) showed interesting experimental properties which prompted its study in man (2,3) (fig 1).

EXPERIMENTAL DATA

As shown in table 1, the concentration which produces a 50% inhibition of the synthesis of DNA in KB cell cultures is 0.25 μg/ml for DTR and 0.15 μg/ml for Adriamycin (ADR). The concentration which inhibits by 50% the multiplication of a DNA virus in tissue culture is 0.130 μg/ml for DTR and 0.045 μg/ml for ADR. The metastatic index (T/C) for Lewis lung carcinoma is especially good for DTR: 0.13 for DTR compared to 0.35 for ADR. In L1210 leukemia the therapeutic index is slightly better for DTR than for ADR. In addition, there are differences in plasma levels, organ fixations, and excretion between the two drugs.

Table 2 shows the ranking of experimental antitumor activity for DTR, daunorubicin (DNR), and ADR; DTR ranks first for most experimental tumors.

The acute toxicity level in terms of mg/kg in mice is lower for DTR than for ADR since the maximum-tolerated dose (MTD) is 14 mg/kg for DTR and 7 mg/kg for ADR (table 3). The subacute toxicity level in mice is also lower for DTR than for ADR since for five consecutive daily treatments the LD50 is between 7.5 and 10 mg/kg for DTR and is 5 mg/kg for ADR. Using the Zbinden test in the rat, the cumulative cardiac toxicity is the same for

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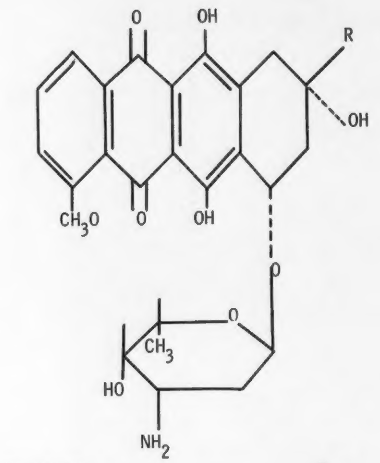

Compound	R
DNR	$-CO-CH_3$
Daunorubicinol (duborimycin)	$-CHOH-CH_3$
Rubidazone	$-\overset{CH_3}{C}=N-NH-CO-C_6H_5$
ADR	$-CO-CH_2OH$
DTR	$-CO-CH_2-O-CO-CH(OC_2H_5)_2$

FIGURE 1.—Some anthracycline derivatives.

both DTR and ADR. The Ames test indicates the same mutagenicity for both drugs and the macrophage toxicity is lower for DTR than for ADR.

Table 4 shows the toxicity of various anthracycline derivatives in terms of LD50 and MTD. DTR is experimentally more toxic than rubidazone and less toxic than ADR.

MATERIALS AND METHODS

The high experimental activity of DTR prompted its study in man. Table 5 shows the distribution of diagnoses for 111 patients who were given this drug at the Hôpital St.-Louis since September 1977. All patients had advanced disease consistent with phase I and II therapeutic trials.

TABLE 1.—Activity of DTR and ADR

Parameter	DTR	ADR
DNA synthesis in KB cells: IC50 (μg/ml)	0.25	0.15
Vaccinia virus multiplication: IC50 (μg/ml)	0.130	0.045
Lewis lung carcinoma (iv treatment), metastatic index (T/C)	0.13	0.35
L1210 therapeutic index	17.5–18.5	14.0–15.0

TABLE 2.—Ranking of DTR, ADR, and DNR according to their antitumor activity in mice*

Tumors	Rank 1	Rank 2	Rank 3
Sarcoma 180	DNR	DTR	ADR
L1210 leukemia	DTR	ADR	DNR
P388 leukemia	DTR	ADR	DNR
AKR lymphoma	DTR	DNR	ADR
Mammary carcinoma	DTR	DNR	ADR
Leucosarcoma	ADR	DTR	DNR

*Compounds were administered iv at the MTD: DTR and DNR, 2.5 mg/kg/day × 5, and ADR, 1.25 mg/kg/day × 5.

TABLE 3.—Toxicity of DTR and ADR

	DTR	ADR
Acute toxicity level (iv), MTD (mg/kg)	14.0	7.0
Subacute toxicity level (iv), LD50 (mg/kg)	7.5–10	5.0
Cardiac toxicity (Zbinden test, $C^tC_{50}D$) (ip)	Same	
Mutagenesis (Ames test)	Same	
Macrophage toxicity	Lower	Higher

The drug doses and schedules varied according to diagnosis as shown in table 6. DTR was given alone by iv infusion in saline. All of the 35 patients with acute leukemia had relapses resistant to previous treatments. DTR was given at a dose of 2 mg/kg/day × 5 or 6 days and the mean total dose was 12 mg/kg. Bone marrow aplasia was usually noted after the tenth day. During aplasia, antibiotic treatment was essential; 30 patients received platelet transfusions and 25 received wbc transfusions from donors with chronic myeloid leukemia. Mucous membrane toxicity was very severe in those patients (oral ulceration and diarrhea) and all subsequent patients with acute leukemia were given intermittent schedules (one 6-mg/kg dose).

The 21 patients with non-Hodgkin's lymphomas had diffuse poorly differentiated lymphoma or diffuse large cell lymphoma (disseminated stage) and all relapses were resistant to CHOP (cyclophosphamide, ADR, vincristine, and prednisone). DTR was given at a dose of 2 mg/kg/day × 2 every 2 weeks or 3 mg/kg every 3 weeks.

TOXICITY

Toxicity is shown in table 7. As previously described, the toxicity for daily DTR administration was prohibitive. For intermediate and intermittent schedules, toxicity was tolerable. Alopecia was observed in all patients.

TABLE 4.—Subacute toxicity in CD_1 mice*

	LD50 (mg/kg)			MTD (mg/kg)		
	Route			Route		
Compound	sc	ip	iv	sc	ip	iv
DNR	11.1	2.0	8.2	2.5	0.7	2.5
Rubidazone	15.0	4.5	7.5	5.0	3.0	5.0
ADR	5.5	3.0	5.0	2.5	0.7	1.2
DTR	7.5	3.5	7.5	2.5	1.2	2.5

*Mice were given a total of 5 treatments on Days 0–4 and were observed during a 21-day period.

TABLE 5.—Diagnoses of patients treated with DTR

Disease	No. of patients
ALL	22
Acute myeloblastic leukemia	7
Acute promyelocytic leukemia	2
Acute monoblastic leukemia	4
Chronic myelocytic leukemia	5
Non-Hodgkin's lymphoma	21
Rhabdomyosarcoma	1
Liposarcoma	1
Hodgkin's lymphoma	4
Epithelioma	21
Malignant melanoma	11
Mesothelioma	1
Osteosarcoma	3
Ewing's sarcoma	1
Parker and Jackson sarcoma	1
Mycosis fungoides	6
Total	111

Nausea and vomiting was observed in one half of the patients. In patients with solid tumors and non-Hodgkin's lymphoma the median wbc count nadir was 1500/mm^3 and the median platelet count nadir was 80,000/mm^3 on the tenth day (median). An electrocardiogram was performed before and after each treatment. In one case cardiac failure appeared after 32 mg/kg of DTR was given within 6 months.

RESULTS

Acute Leukemia

Response to treatment was evaluated by the criteria of the Acute Leukemia Cooperative Group B (4). The results of treatment in acute leukemia are summarized in table 8. Thirteen (59%) of 22 patients with acute lymphoblastic leukemia (ALL) had complete remissions (CRs). Ten (76%) of the 13 patients in their first relapse achieved CRs. Three (38%) of eight patients in their second relapse achieved CRs. Five patients were resistant to DTR. Three deaths occurred during the induction treatment while the patients had bone marrow aplasia caused by the first course of the drug. The duration of CR was short (median, 20 days). In a child with Burkitt's lymphoma resistant to the combination of prednisone, vincristine, cyclophosphamide, and DNR, one single dose of 6 mg/kg of DTR achieved a complete hematologic remission.

In patients with acute granulocytic leukemia four (30%) of 13 patients achieved CR: one of seven patients with acute myeloblastic leukemia, two of four patients with acute monoblastic leukemia, and one of two patients with acute promyelocytic leukemia. One of the patients with acute monoblastic leukemia with a complete hematologic remission had a skin relapse which was assessed histologically and two skin nodules disappeared after a total dose of 4 mg/kg of DTR. The CRs were of short duration

TABLE 6.—DTR treatment: dosage and schedule of administration

Disease	Dosage (iv)	Mean total dose
Acute leukemia (35 patients)	2 mg/kg/day × 5 days then stop	12 mg/kg
Non-Hodgkin's lymphomas (21 patients)	2 mg/kg/day × 5 days every 15 or 21 days	10 mg/kg
Solid tumors (55 patients)	1 mg/kg × 1 wk, 2 mg/kg/day every 3 wks	8 mg/kg

TABLE 7.—DTR toxicity

Administration schedule	Diagnosis	Total No. of patients	No. of patients with toxicity*			
			Hematologic		Mucous membrane	
			1–2	3–4	1–2	3–4
Daily	Acute leukemia	35		35	11	17
Intermediate	Non-Hodgkin's lymphoma	21	7	9	5	4
Intermittent	Epithelial melanoma	55	12	8	8	1

*1–2 = mild to moderate toxicity and 3–4 = severe toxicity.

TABLE 8.—DTR response in acute leukemia*

	No. of patients with—			
	ALL	Acute myeloblastic leukemia	Acute monoblastic leukemia	Acute promyelocytic leukemia
CR	13 (59%)	1	2	1
PR	1	—	—	1
Failure	5	5	1	—
Death during aplasia	3	1	1	—
Total	22	7	4	2

*All patients were in relapse except for one with ALL who had failed to respond to prior treatment (DTR).

TABLE 9.—Effect of DTR on non-Hodgkin's lymphoma

	No. of patients		
	CR + PR	Failure	Total
No prior treatment	4	—	4
Prior treatment*	11†	6	17
Total	15 (71%)	6	21

*Patients resistant to CHOP.
†4 patients with skin involvement.

and mucous membrane toxicity (oral ulcerations) was severe in these patients.

The results have been especially encouraging in non-Hodgkin's lymphoma patients (table 9). Fifteen (71%) of 21 patients achieved remission. The activity of DTR on skin involvement appears especially striking. In these advanced-disease patients, remissions were of short duration. However, the achievement of remission after one or two doses of DTR in these rapidly progressive diseases resistant to all other drugs emphasizes the major activity of this new derivative and suggests that randomized studies be done in non-Hodgkin's lymphomas with ADR versus DTR.

A CR was obtained in one child with rhabdomyosarcoma with bone marrow involvement and with bone, liver, and pulmonary localization. The duration of CR was 6 months.

There was a > 50% regression of disease in five patients with mycosis fungoides who received low-dose (1 mg/kg/week) DTR. A striking shrinkage of an abdominal liposarcoma was also observed and surgery was made possible by treatment with DTR. A CR was observed in one case of peritoneal mesothelioma which was resistant to radiotherapy.

Five patients with bone sarcoma received DTR. A CR was achieved in a patient with Parker and Jackson sarcoma resistant to previous treatment (including ADR and actinomycin D). A complete disappearance of bone pain was observed in two patients with osteosarcoma who received one dose of DTR before amputation.

Table 10 summarizes the results of treatment of carcinomas with DTR. Among 21 patients, we observed 11 good results including > 50% regression in three patients: one with lymph node metastasis from a thyroid carcinoma, one with skin metastasis from renal carcinoma, and the other with bone metastasis. There were three partial regressions (PRs) and one CR lasting for 7 months among 11 patients with disseminated malignant melanoma resistant to chemotherapy.

TABLE 10.—Results of DTR treatment of 21 patients with carcinomas

Tumor type	Total No. of patients	No. of patients with— > 50% regression	< 50% regression	Failure
Breast	3		3	
Thyroid	2	1	1	
Kidney	2	1		1
Ovary	2		1	1
Primary unknown	4	1	3	
Miscellaneous	8			8*

*1 patient death during aplasia.

CONCLUSION

DTR has a wide range of activity in malignant diseases. In acute leukemias, daily administration proved active but too toxic. In non-Hodgkin's lymphomas, intermittent administration led to a 71% CR rate in advanced disseminated disease with poor histologic prognosis. Skin involvement proved to be especially sensitive to this therapy. DTR may be useful at low doses in advanced stages of mycosis fungoides. Its activity is striking in soft tissue sarcomas and is encouraging in melanomas. It may also be active in the treatment of various other types of cancers. The elective affinity of DTR for skin involvement raises interesting questions concerning metabolism, plasma levels, organ fixations, and excretion. Experimental data confirmed differences between DTR and other anthracycline derivatives.

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Clinical Studies With Daunorubicin-DNA and Adriamycin-DNA Complexes: A Review

A. Trouet* and G. Sokal[1]

SUMMARY

The clinical results presently available on the use of daunorubicin (DNR)-DNA and Adriamycin (ADR)-DNA are reviewed in light of the most recent experimental data regarding the anthracycline-DNA complexes. Three randomized trials indicate that ADR-DNA is superior to DNR-DNA in combination therapy for acute lymphoblastic leukemia, that DNR-DNA equals DNR in the treatment of acute nonlymphoblastic leukemia, and that ADR-DNA might be equal to ADR in the chemotherapy for anaplastic bronchogenic carcinoma. Increasing evidence suggests that the anthracycline-DNA complexes are less cardiotoxic than the corresponding free drugs.

[Cancer Treat Rep 63:895–898, 1979]

The use of anthracycline-DNA complexes was first proposed in 1972 (1), on the basis of the drug-carrier concept, as a way to reduce the toxicity and increase the therapeutic efficiency of daunorubicin (DNR) and Adriamycin (ADR). The published results from trials using DNR-DNA and ADR-DNA in the treatment of human malignant diseases will be reviewed here. In order to put these results in their correct perspective, we will summarize first the most recent data obtained with the DNA complexes in experimental animal models.

EXPERIMENTAL DATA

We have shown recently that DNR-DNA is not a stable complex in the bloodstream after an iv injection (2,3). DNR is very quickly released from its carrier, and the DNR-DNA complex must be considered more as a slow release form of DNR endowing DNR with specific pharmacokinetic and tissue distribution properties. The plasma levels of DNR, reached after administration of DNR-DNA, are indeed significantly increased while the uptake of DNR in tissue such as the heart is decreased (3). The slow release properties of DNR-DNA may explain why its toxicity is not decreased in mice after iv injection.

In contrast to DNR-DNA, ADR-DNA is much more stable in the bloodstream (2,3) and fulfills, in a much better way, the requirements of an ideal drug-carrier complex (4). ADR and DNA are cleared from the plasma in a nearly parallel manner and the plasma levels of ADR are very much increased. This stability of the ADR-DNA complex may explain why it is less toxic after iv injection than ADR even in the presence of higher drug levels and why the chemotherapeutic efficiency of ADR-DNA in L1210 leukemia is enhanced more than that of DNR-DNA (2,3).

The uptake of ADR in the heart after ADR-DNA injection is also very significantly decreased by a factor of 2 to 3, both in mice (2,3) and in rats (J. C. Blanchard et al, manuscript in preparation). These results are in agreement with the data of Langslet et al (5), who showed clearly that the acute cardiotoxicity of ADR can be decreased by binding to DNA, and tend to confirm experimentally our starting hypothesis that the uptake of ADR by the heart should be reduced when combined with high molecular weight DNA (6).

The increased chemotherapeutic activity of DNR-DNA has been confirmed by other investigators (7,8); it is, however, clear that such an enhancement is more striking with ADR-DNA than with DNR-DNA and that it is only obtained in tumor cell lines which are sensitive to the free drugs. Atassi et al (9) have illustrated this clearly by using two different L1210 cell lines. The first one ($L1210_T$), which is sensitive to ADR when injected in DBA_2 mice, is much more sensitive to ADR-DNA while the same cell line injected in BDF_1 mice becomes much less sensitive to ADR and does not demonstrate as well the greater sensitivity to ADR-DNA. Similar results were obtained with the $L1210_B$ cell line, which is also less sensitive to ADR, in both DBA_2 and BDF_1 mice. We are tempted to conclude from these results that the binding of anthracyclines to DNA does not overcome a resistance of tumor cells to the free drugs.

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Although ADR-DNA is superior to ADR in leukemic animal malignancies (iv inoculated L1210 leukemia), it seems that its activity is equal to that of ADR in solid tumors like C3H mouse transplanted mammary adenocarcinoma (10), Lewis lung carcinoma (11), and Madison 109 tumor (8). A low activity of ADR-DNA has been found by Atassi et al in B16 melanoma and L1210 leukemia implanted sc (11). By using another iv administration schedule, we could, however, observe superior activity of ADR-DNA in sc implanted L1210 leukemia as compared to ADR (2).

From these experimental data one could provisionally conclude that the activity of anthracycline-DNA complexes is increased in leukemic forms of disease which are sensitive to the free drugs. An increased effectiveness of DNA complexes against solid tumors cannot always be shown and should be further investigated. Even an equal activity of the DNA complexes would, however, justify their use in view of the decreased uptake of the anthracycline by the heart when given as a DNA complex.

CLINICAL DATA

Pharmacokinetic studies performed in man with DNR-DNA and with ADR-DNA (12,13) confirm the data obtained in animals. Plasma levels achieved with the iv administration of DNR and ADR as DNA complexes are significantly increased in terms of total fluorescence as compared to the free drugs. These results suggest very strongly that the concentration-×-time parameter of ADR-DNA and DNR-DNA is significantly higher than that obtained with the free drugs.

The clinical activity of DNR-DNA was first investigated in combination with vincristine (VCR) in acute nonlymphoblastic leukemia (ANLL). A complete remission (CR) rate of 58% was obtained in previously treated patients (14). Combination chemotherapy regimens including ADR-DNA also yielded promising results in other tumor types. Seven CRs were obtained with ADR-DNA, VCR, and prednisone in 11 patients with lymphosarcoma (15). Responses were also seen with ADR-DNA, VCR, and medroxyprogesterone in a small series of patients with breast cancer resistant to a four-drug regimen (16).

Lie et al (17) have tested ADR-DNA in a variety of childhood tumors and leukemias. They observed spectacular regressions of metastases in three cases of Wilms' tumor, one case of testicular embryonic carcinoma, and one case of neuroblastoma. They have treated 16 children with ANLL with a combination of ADR-DNA and cytosine arabinoside and have observed 14 CRs, with three children no longer receiving therapy; the longest observation time has been 4 years.

In a first randomized trial, Ferrant et al (18) have compared the effectiveness of DNR-DNA and ADR-DNA combined with L-asparaginase, prednisone, and VCR in 69 children with acute lymphoblastic leukemia (ALL). They observed significantly better results with the ADR-DNA regimen, with a 5-year survival rate of 74.5% versus 38% with the DNR-DNA regimen (P = 0.03).

In a second trial, these investigators randomized 26 patients with ANLL between DNR and DNR-DNA in combination with cytosine arabinoside (18). The results indicated that DNR and DNR-DNA have an equivalent effectiveness. The 1-year survival rate was 66% for the DNR group and 64% for the DNR-DNA group. Similar preliminary observations were reported by Gahrton et al (19)

Bosly et al (20) have randomized 51 patients with anaplastic bronchogenic carcinoma between ADR and ADR-DNA. There were no significant differences observed between the remission rates of patients treated with ADR-DNA (11 of 25 patients) and those treated with ADR (17 of 26 patients), although there seems to be a trend in favor of ADR alone. The 1-year survival rates of the two groups were very similar (17.5% for ADR and 11.5% for ADR-DNA).

All previously mentioned reports agree with the conclusion that the toxic side effects such as alopecia, stomatitis, and bone marrow depression observed with DNR-DNA and ADR-DNA are quite comparable to those observed with the free drugs. The DNA complexes are well-tolerated provided the perfusions are performed at a slow rate and, as shown for the first time by Lie et al (17), only freshly autoclaved DNA is used. Allergic anaphylactic reactions or the occurrence of anti-DNA antibodies has not been reported.

There is an increasing suggestion that the cardiotoxicity of the DNR-DNA and ADR-DNA complexes is reduced when compared to the free drugs. Table 1 shows the total amount of DNR or ADR which was given either totally or partially as the DNA complex to 588 patients treated at the Université Catholique de Louvain. As can be seen, 63 patients have received a cumulative dose of > 550 mg/m^2 of ADR or 750 mg/m^2 of DNR. Thus far, none of these 588 patients has developed congestive heart failure during therapy with either complex.

Similar observations were made by Lie et al (17) who reported that in 20 children who received > 500 mg/m^2 of ADR-DNA no clinical signs of cardiotoxicity related to the administration of ADR could be found. In 16 of

Table 1.—Dose classification of patients treated with DNR- and ADR-DNA complexes (with or without prior therapy with free drugs)*

Drug	No. of patients receiving total doses (mg/m²) of—					Total No. of patients
	< 250	250–549	550–749	750–999	> 1000	
DNR-DNA	64	48	17	8	1	138
DNR + DNR-DNA	3	1	2	0	0	6
ADR-DNA	259	78	20	16	1	374
ADR + ADR-DNA	7	11	8	8	1	35
ADR-DNA + DNR-DNA	11	20	4	0	0	35
Total	344	158	51	32	3	588

*These patients were treated by Drs. Sokal, Maisin, and Prignot at the Université Catholique de Louvain.

these patients, detailed pathologic examinations of the heart after death did not reveal any sign of cardiac damage. Two patients with congestive heart failure and one patient with an abnormal heart biopsy were reported by Benjamin et al (21) after administration of ADR-DNA to 58 patients. It was concluded that cardiotoxicity was similar with ADR-DNA and ADR but the two patients with congestive heart failure received prior therapy with free anthracyclines. In addition, as pointed out by these authors, the stability of the complex could not be reliably ascertained.

CONCLUSIONS

This overview of clinical data supports the conclusion that DNR-DNA and ADR-DNA have an activity at least equal to that of the free drugs in the treatment of leukemia. DNR-DNA is as active as DNR in ANLL and ADR-DNA is superior to DNR-DNA in ALL. ADR-DNA has very good activity in ANLL but has not yet been compared in a randomized trial with ADR.

Anecdotal results indicate that ADR-DNA is active against a variety of hematosarcomas and solid tumors such as Wilms' tumor, neuroblastoma, and breast carcinoma. One randomized trial has shown ADR-DNA to be as active as ADR in the treatment of bronchogenic carcinoma.

Increasing evidence suggests that the cardiotoxicity of ADR-DNA is reduced as compared to ADR. ADR-DNA is probably not totally devoid of cardiotoxicity but the experimental data showing a two- to three-fold decreased uptake of ADR by the heart is consistent with a shift in the critical cumulative dose of ADR-DNA to a value varying between 1000 and 1500 mg/m². The apparently decreased cardiotoxicity of the DNA complexes warrants further investigation in confirmatory clinical trials.

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American Experience With Carminomycin

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Goals of new anthracycline development include (*a*) a broader spectrum of activity and reduced toxicity (cardiac and myelosuppressive); (*b*) oral activity; and (*c*) decreased cost. Carminomycin is a Russian-developed drug that is reported to have some, if not all, of these qualities. Carminomycin was isolated from *Actinomadura carminata* in the USSR. The drug appears to be biochemically closely related to Adriamycin and chemically related to daunorubicin (*see* fig 1) (1).

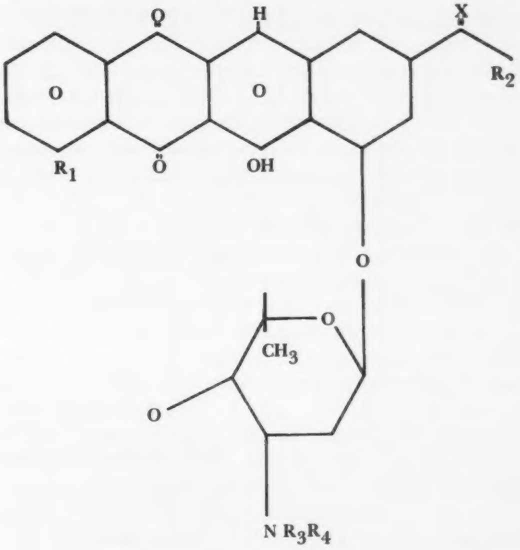
Clinically pertinent data from Russian studies (2,3)[5] indicate that carminomycin is active against soft tissue sarcomas, lymphomas, and acute leukemia. Additionally, preliminary data suggest activity against breast, gastric, ovarian, and corpus uterine cancers (table 1). Two dose schedules have been employed: 7.5 mg/m^2 twice weekly and 5.5 mg/m^2/day × 5 days (table 2). Toxic effects reported with these studies included myelosuppression (predominantly leukopenia) and nausea and vomiting (4). Of potential importance is the report that the incidence of alopecia was low and that congestive heart failure was not seen (table 3).

Studies with carminomycin in the US have thus far been limited to animal toxicity studies conducted at Bristol Laboratories and two phase I studies, both of which are not yet complete. We are now conducting a phase I trial using a single intermittent bolus iv dose at Upstate Medical Center and a phase I study using an oral single-dose intermittent schedule at Wayne State University.

TOXICITY

Studies with single- and multiple-dose iv schedules in beagle dogs demonstrate that the toxicity of carminomycin is dose-related and primarily manifested by its suppressing action on hematopoiesis (table 4).[6] Histologic examination showed that dogs treated twice weekly at relatively high doses (2.4–5.0 mg/m^2) for 3 weeks had a reduction in the numbers of bone marrow cells of myeloid and erythroid series, of splenic lymphoid and/or reticuloendothelial cells, and of lymphoid cells in other lymphoid tissues.

Preliminary studies employing the Zbinden rat model (5) showed that carminomycin produced less cardiac damage as evidenced by less focal necrosis characteristic of Adriamycin-induced histologic changes when the two drugs were administered at doses which produced comparable lethality. The groups of rats which received car-



	R$_1$	R$_2$	R$_3$	R$_4$	X
Adriamycin	OCH$_3$	CHOH	H	H	O
Carminomycin	OH	CH$_3$	H	H	O
Daunorubicin	OCH$_3$	CH$_3$	H	H	O
Rubidazone	OCH$_3$	CH$_3$	H	H	NNHCOO

FIGURE 1.—Chemical structures of Adriamycin, carminomycin, daunorubicin, and rubidazone.

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TABLE 1.—Response rates to iv carminomycin

	No. of patients	No. of responses	Response rate (%)
Solid tumors			
Soft tissue sarcoma	48	13	27
Osteogenic sarcoma	8	1	12.5
Breast carcinoma	37	7	19
Stomach carcinoma	15	2	13
Ovarian carcinoma	16	2	12.5
Uterine carcinoma	3	1	33
Kidney carcinoma	5	0	0
Lung cancer	17	0	0
Malignant melanoma	7	0	0
Total	156	26	17
Lymphomas			
Hodgkin's disease	6	3	50
Reticulosarcoma	18	9	50
Total	24	12	50
Acute leukemia	48	13	27
Overall total	228	61	22

TABLE 2.—Dose schedules of carminomycin in adults: USSR studies

	iv dose (mg/m^2)	
Schedule	Per day	Per course
Twice weekly × 3 wks, repeated every 4 wks	7.5	35–45
Daily × 5, repeated every 3 wks	5.5	23–30

TABLE 3.—Toxicity reported with iv carminomycin in a cooperative USSR study

Toxic effect	% of patients
Leukopenia	43.4
Thrombocytopenia	8.4
Nausea and vomiting	18.3
Electrocardiographic changes	5.4
Other	6.6

TABLE 4.—Acute iv toxicity in dogs

Single iv dose (mg/kg)	No. of dogs treated	Sex	No. of deaths	Time of deaths (days after dosing)	Toxic effects
0.8	2	M	2	11, 12	Emesis, decreased activity, decreased appetite, enlarged tonsils, diarrhea, and convulsions before death
	2	F	2	7, 9	
0.6	2	M	2	11, 11	Emesis, decreased activity, decreased appetite, inflammed tonsils, and convulsions before death
	2	F	0	—	
0.4	2	M	0	—	Rarely disturbed appetite, slight weight loss, and slightly inflammed tonsils in 1 of 4 dogs
	2	F	0	—	
0.2	2	M	0	—	Emesis in 1 of 4 dogs and some disturbance of appetite in 2 of 4 dogs
	2	F	0	—	
0.05	2	M	0	—	None
	2	F	0	—	
Control (sterile water)	2	M	0	—	None
	2	F	0	—	

TABLE 5.—Oral toxicity levels in dogs

Toxicity level	Dose	
	mg/kg	mg/m²
Highest nontoxic dose	0.8	16.0
Toxic dose low (range)	0.8–1.5	16–31
Toxic dose high (range)	1.5–2.4	31–48
Lethal dose (range)	1.5–2.4	31–48
LD50	2.7	54

minomycin also had fewer electrocardiographic changes than Adriamycin-treated animals.

Studies of acute oral toxicity were conducted with dogs and monkeys which showed that the dog was the more sensitive species. The results of this acute oral toxicity study in dogs can be seen in table 5. These levels were used as the basis for the initial dose in the oral phase I study.[6]

CLINICAL STUDIES

Our clinical studies are still incomplete, but some pharmacokinetic data are available (table 6). The assay system can detect a 10-ng/ml concentration of anthracycline in plasma using a combination of organic solvent extraction and thin-layer chromatography on silica and fluorescence detection. Only the aglycone of carminomycin was detected in the plasma of four patients who had received a 24-mg/m^2 drug dose (peak levels, 185–600 ng/ml). Free carminomycin (peak level, 39 ng/ml) was detected in the plasma of a patient who received 36 mg/m^2 of drug.

Thus far, no quantitative toxicity has been encountered with oral doses ranging from 3 to 36 mg/m^2. Three patients have experienced mild gastrointestinal upset. No patient as yet has experienced myelosuppression, alopecia, or cardiac abnormalities not attributable to other more obvious causes.

The iv phase I study has not reached the maximally tolerated dose; however, two of three patients treated at a dose of 15 mg/m^2 experienced wbc counts of < 3000/mm^3 2 weeks after drug administration.

The Russian experience with carminomycin suggests that this drug has a broad spectrum of activity with less toxicity than Adriamycin. Initial confirmation studies are in progress in the US.

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[6] Carminomycin hydrochloride toxicologic studies. Bristol Laboratories, July 21, 1977.

TABLE 6.—Oral phase I study of carminomycin

Dose level (mg/m^2)	No. of patients	No. of courses	Comment
3	3	5	—
6	2	2	In 1 patient 20 ng/ml of aglycone was detected 3 hrs after administration
12	3	5	—
24	3	4	All drug detected was aglycone; mean peak = 442 ng/ml; mean time of peak = 3.4 hrs; mean time to disappearance = 16 hrs
36	1	2	Free carminomycin detected; peak = 39 ng/ml at 1 hr

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Early Clinical Trial With Quelamycin

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SUMMARY

Quelamycin (triferric doxorubicin) is a derivative of Adriamycin with different pharmacologic properties. Our phase I clinical study of quelamycin includes 37 patients with a wide spectrum of solid tumors. The recommended dose in good-risk patients is 150 mg/m², given as a 1-hour infusion every 3 weeks. The dose-limiting factor appears to be myelosuppression, especially leukopenia. Other toxic effects include gastrointestinal intolerance and alopecia. Chills and fever are commonly encountered and might be due to an excess of free iron in currently available preparations. Cardiotoxicity could not be properly assessed. An objective antitumor effect was seen in patients with lung, gastric, colon, and ovarian carcinomas as well as osteogenic sarcoma. Further preclinical and clinical studies with an improved pharmaceutic formulation of the drug are highly desirable.

[Cancer Treat Rep 63:903–908, 1979]

Quelamycin is a derivative of Adriamycin developed by Gosálvez (1) (fig 1). The drug is prepared by neutralizing an aqueous solution of Adriamycin and ferric chloride with NaOH. For clinical use, quelamycin is buffered with tris aminomethane in 5% mannitol. This product is filtered twice through 0.2-μ Millipore filters before deep freezing and lyophilization. A fast-freezing procedure is necessary to reduce the polymerization of the drug. In this paper, a 1-mg equivalent of quelamycin is defined as the quantity of quelamycin resulting from the chelation of 1 mg of Adriamycin.

Binding of ferric ions to Adriamycin significantly modifies the properties of this drug in terms of cell uptake, distribution, and metabolism (2). The LD50 in mice is approximately three times greater with Adriamycin than with quelamycin (3). Initially, quelamycin was developed rationally as a potentially non-cardiotoxic anthracycline derivative. The cardiotoxicity induced by Adriamycin has been possibly related to its inhibiting effect on the heart Na-K ATPase (4). This inhibition has not been detected with quelamycin (3), whereas its experimental antitumor activity remains similar to that achieved with Adriamycin (fig 2).[3]

Histopathologic evidence of cardiomyopathy has been demonstrated in rats and rabbits following repeated administration of quelamycin (5). The incidence of cardiac damage was similar to that observed with Adriamycin at total cumulative doses that were approximately twofold greater with quelamycin than with Adriamycin. In these experiments, a possible iron-induced cardiotoxicity (6) cannot be ruled out, especially since free iron may be

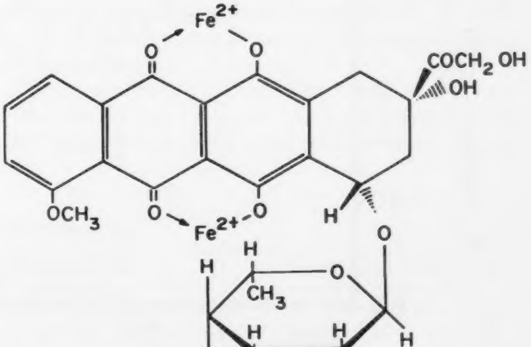

FIGURE 1.—Chemical structure of quelamycin. The chelation sites of Adriamycin were determined by spectral analysis at low iron concentrations in neutral solutions of D_2O (3).

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present in current pharmaceutic formulations. Our phase I clinical trial with quelamycin was initiated in 1977. Preliminary results of this study have been reported elsewhere (7).

MATERIAL AND METHODS

All patients entered in the study had histologic evidence of solid malignant tumors for which no therapy of proven clinical benefit was available. Selection criteria also included a life expectancy of > 8 weeks, a Karnofsky performance status of > 30%, and no chemotherapy or radiotherapy in the past 12 weeks. Four patients had received Adriamycin previously. All patients had normal cardiac (clinical and electrocardiographic) function, > 5000 wbcs/mm^3, > 100,000 platelets/mm^3, serum creatinine $\leq$ 2.0 mg/100 ml, SGOT $\leq$ 100,000 IU, and bilirubin $\leq$ 2.5 mg/100 ml. Blood cell counts were repeated every other day in hospitalized patients and weekly in outpatients. Physical examination, electrocardiogram (ECG), chest roentgenogram, performance status, and tumor measurement were recorded before each cycle of therapy.

Quelamycin was supplied and manufactured by M. Gosálvez at Montedison Farmaceutica (Barcelona, Spain) in vials containing a black lyophilized powder of 25-mg equivalent of quelamycin. Each vial of quelamycin was reconstituted with 10 ml of twice-distilled water for iv injection. Quelamycin was administered as a slow iv infusion for at least 1 hour every 3 weeks. The starting dose was 75 mg/m^2 in all but the last seven patients who initially received 125 mg/m^2. In subsequent courses the dose was escalated by progressive increments of 25 mg/m^2 until the occurrence of limiting toxicity. A minimum wbc count of 3000/mm^3 was required before giving the next course. Escalation was made if the nadir leukocyte count was not < 2000/mm^3 or if the nadir platelet count was not < 100,000/mm^3 during the previous course. The dose was reduced if patient tolerance was poor or toxicity was severe.

Objective response to therapy was defined as a decrease by $\geq$ 50% in the product of the two largest perpendicular diameters of measurable lesions. Stable disease corresponded to a decrease by < 50% or unchanged lesions. Progressive disease was defined as any increase in the size of lesions or the appearance of new lesions.

Limited pharmacokinetic studies were carried out.

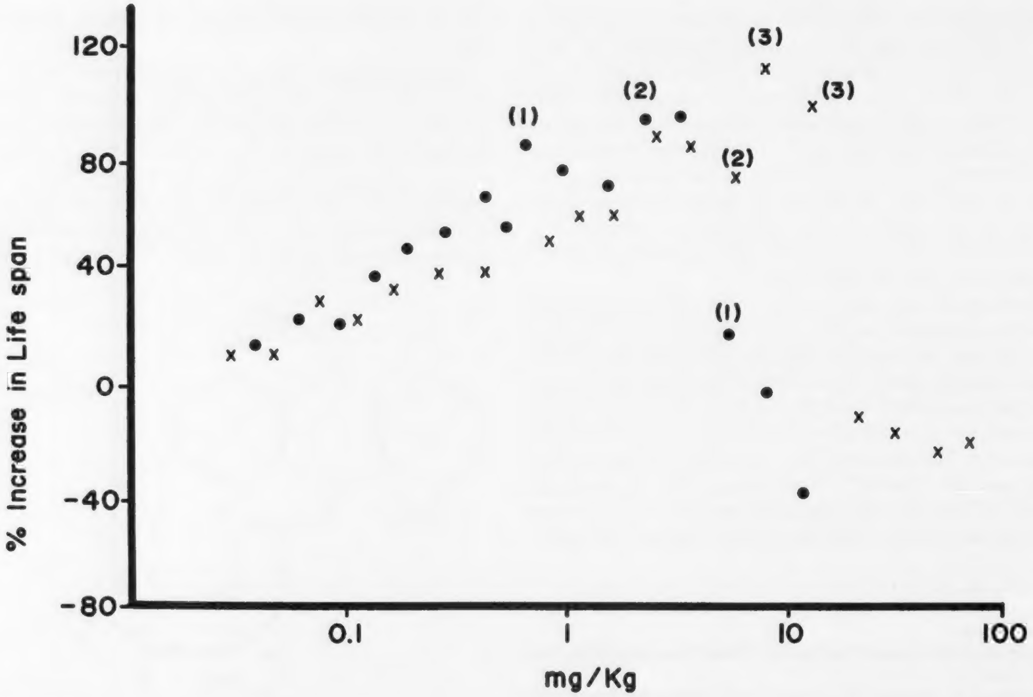

FIGURE 2.—Increase in lifespan as measured by the median survival time in groups of 8 mice bearing P388 leukemia treated ip with different doses of Adriamycin (circles) or quelamycin (crosses) administered ip repeatedly every 4 days, Days 1, 5, and 9. Nos. in parentheses are No. of survivors at Day 25 (*see* footnote 3).

Levels of quelamycin were determined in the plasma, urine, and cerebrospinal fluid (CFS) of a few patients. Drug levels were assayed by total fluorescence of Adriamycin after dissociation of the iron ions by treating the samples with 0.45 N HCl solution. Fluorescence peaks of Adriamycin were selected at 470-nm wavelength (excitation) and 585-nm wavelength (emission) (8).

RESULTS

Forty-eight patients were entered in the study but 11 were not evaluable: six patients died of progressive disease within 3–4 weeks of entry and five patients were lost to followup. Among the 37 evaluable patients, there were 21 men and 16 women with a mean age of 46 years (range, 16–68 years). Progressive disease occurred in 22 patients after chemotherapy, in three after radiotherapy, and in 12 who had no prior therapy. Three patients with breast cancer and one patient with osteogenic sarcoma had previously received Adriamycin without response. A total of 112 courses of therapy were administered. Only one patient received eight cycles of therapy, two patients received six cycles, six patients received five cycles, nine patients received four cycles, eight patients received three cycles, and one patient received two cycles.

Toxicity

Hematologic toxicity was dose-related and dose-limiting (table 1). It consisted mainly of leukopenia and, to a much lesser degree, thrombocytopenia; hemoglobin levels were not affected. Acute toxicity appeared 30 minutes to 4 hours after the administration of quelamycin (table 2). It consisted of nausea and vomiting, fever and chills, and malaise. Nausea and vomiting were always mild, starting at the end of the infusion and lasting up to 24 hours. Chills and fever (38°C–40°C) started during the infusion. Complete recovery occurred spontaneously within 1 or 2 hours. Malaise, which was always mild, started during the infusion and could last up to 1 week.

Alopecia was universal and generally started after the second cycle. Mental stupor was seen for several days in one patient and disappeared spontaneously. Another patient, with pulmonary metastasis of breast cancer, had some discrete dypsnea with asthmatic symptoms immediately following the injection. A third patient developed thrombophlebitis at the site of the injection. Six patients complained of a metallic taste after the drug infusion. Following the infusion of the drug, the urine was red in 18 patients and violet in the others.

Low-voltage ECG with hypertension after 475 mg/m^2 of the drug was seen for about 2 weeks in one patient. This patient, who had previously received 550 mg/m^2 of Adriamycin, had shown similar signs with the last injection of Adriamycin. The interval between Adriamycin and quelamycin treatments was > 8 months. Another patient exhibited signs of subepicardiac ischemia on ECG after receiving a cumulative dose of 375 mg/m^2. The ECG normalized after 32 days. No acute or cumulative cardiotoxicity was noted in the other patients. Four patients received doses of quelamycin > 550 mg/m^2 (600, 700, 750, and 1700 mg/m^2). The patient who received 1700 mg/m^2 died of tumor progression, and no microscopic evidence of cardiomyopathy was found at postmortem examination. At autopsy electron microscopy failed to reveal any sign of cardiomyopathy in the patient who received 700 mg/m^2.

No liver or renal toxicity could be identified. The sympathomimetic crisis described by Brugarolas et al (9), with facial flushing, sweating, thirst, chest pains, backache, bronchospasm, coughing, shortness of breath, tachycardia, headache, and tremors, was not encountered in any of our patients. No toxic deaths occurred in our study.

Pharmacokinetics

Eight patients had serial measurements of plasma and urine concentrations of quelamycin after having received 125 mg/m^2 of the drug (fig 3). In two of these patients, quelamycin concentrations were measured in the CSF. No free Adriamycin was detected in any samples before iron dissociation.

The average plasma concentration 1 hour after the end of the infusion was 1.8 μg/ml; this concentration diminished to an average of 0.4 μg/ml after 24 hours. The cu-

TABLE 1.—Hematologic toxicity of quelamycin

Dose (mg/m^2)	No. of patients	No. of evaluable courses/total courses given	Median day of wbc count nadir	No. of patients with wbc count (× 1000/mm^3) of—					No. of patients with platelet count (× 1000/mm^3) of—		
				4	3–4	2–3	1–2	< 1	75–100	75–50	< 50
75	30	38/38	14	13	14	5	2	4	—	1	1
100	9	9/9	13	2	5	2	—	—	—	1	—
125	20	22/26	13	2	6	6	4	4	1	—	—
150	22	18/30	14	2	6	3	5	2	—	—	—
200	6	8/9	11	—	6	—	2	—	—	—	—

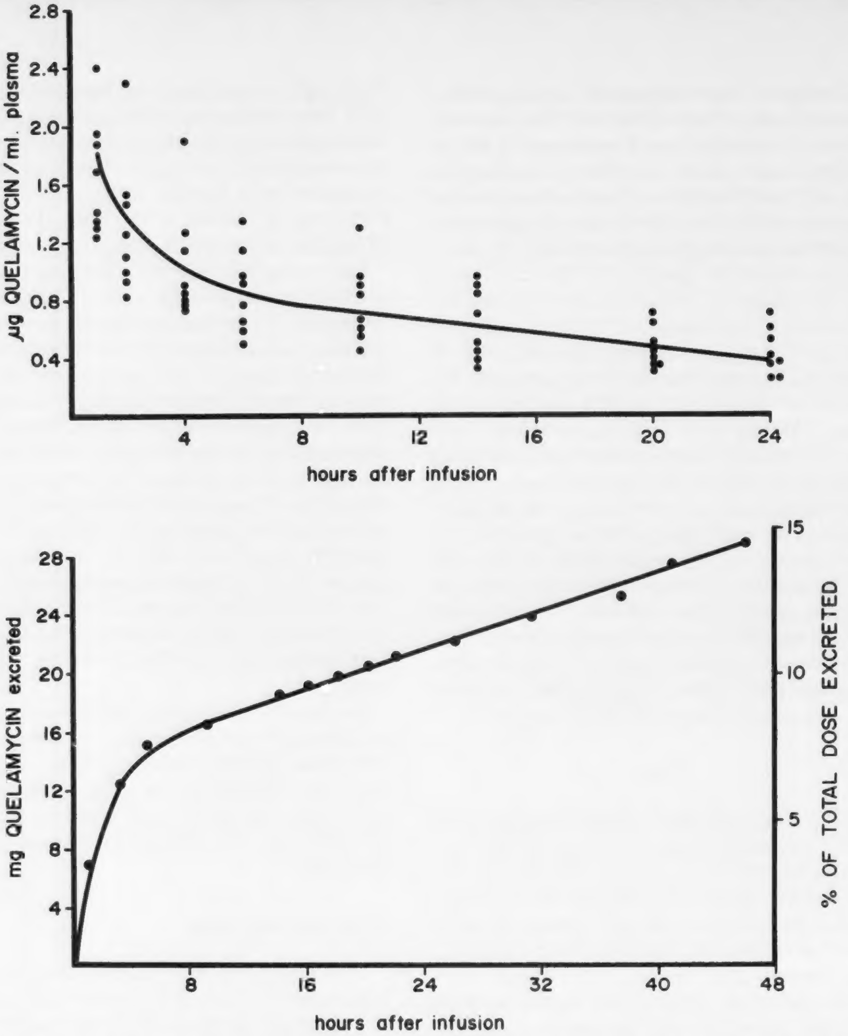

FIGURE 3.—(*Top*) Concentrations of quelamycin in the plasma of 8 patients following the end of an iv infusion of 125 mg/m^2. (*Bottom*) Cumulative excretion of quelamycin in the urine of 1 patient following an iv infusion of 125 mg/m^2.

TABLE 2.—Toxic effects (other than myelosuppression) after the first course of quelamycin

Toxicity	No. of patients with toxicity/ total No. treated	%
Malaise	37/37	100
Alopecia	37/37	100
Chills and fever	32/37	86.5
Red urine	18/37	48.6
Metallic taste	6/37	16.2
Confusion	1/37	2.7
Dypsnea	1/37	2.7
Thrombophlebitis	1/37	2.7
Stomatitis	0/37	0
Sympathomimetic reaction	0/37	0

mulative urinary excretion during 48 hours amounted to 15% of the total dose received by the one patient in whom urinary recovery could be determined. Quelamycin was not found in the collected CSF.

Response

Objective responses were observed in seven of the 37 evaluable patients (18.9%) (table 3): three with lung cancer, one with gastric carcinoma, one with osteogenic sarcoma, one with colon carcinoma, and one with ovarian carcinoma. Responses were achieved with the first course of therapy and apparently did not increase with subsequent courses. Stabilized disease was observed in six other patients. Among these, three patients had proven to be resistant to prior Adriamycin therapy. All responses were maintained for > 2 months and the longest response (7 months) was obtained in a patient with lung carcinoma.

DISCUSSION

In addition to the nausea, vomiting, bone marrow depression, and alopecia which are commonly produced by Adriamycin, quelamycin may also induce transient

TABLE 3.—Objective responses to quelamycin

Diagnosis	No. of patients	No. of responses		
		Partial response	Stable disease	Progressive disease
Lung carcinoma				
Adenocarcinoma	3			3
Epidermoid	9	1	2	6
Anaplastic	1	1		0
Oat cell	2	1		1
Breast carcinoma	6		2*	4
Gastric carcinoma	6	1	1	4
Osteogenic sarcoma	1	1*		0
Leiomyosarcoma	1		1	0
Renal carcinoma	2			2
Carcinoma, unknown origin	2			2
Epidermoid carcinoma, maxillary	1			1
Pancreatic carcinoma	1			1
Colon carcinoma	1	1		0
Ovarian carcinoma	1	1		0
	37	7	6	24

*Tumors resistant to Adriamycin.

chills and fever. Chills and fever are frequently encountered following iv administration of iron (6) and could probably be reduced with improved pharmaceutic formulations of quelamycin containing either no or smaller amounts of free iron.

The dose-limiting factor of quelamycin appeared to be hematologic toxicity and the recommended therapeutic dose for good-risk patients is 150 mg/m^2, given as a 1-hour iv infusion every 3 weeks. In our series of patients, we have not encountered the sympathomimetic crisis described by Brugarolas et al (9) who used a more rapid infusion.

Cumulative heart toxicity was not documented. According to the recent analysis of Von Hoff et al (10), Adriamycin cardiotoxicity is a relatively rare event and only a large series of patients having received large amounts of quelamycin could answer the question of reduced long-term cardiotoxicity.

Our pharmacokinetic data, although preliminary, show differences relative to Adriamycin. The practical implications of these observations remain to be determined.

Objective responses were observed in various tumor types, which may indicate that the drug has a wide spectrum of activity. Of note, a response was also achieved in a tumor resistant to Adriamycin. This apparent lack of cross resistance between Adriamycin and quelamycin has also been reported by others (9).

In conclusion, quelamycin has shown antitumor activity and tolerable toxicity as well as differences in pharmacologic disposition and perhaps lack of cross-resistance with Adriamycin. These findings warrant further investigation especially with an improved pharmaceutic formulation of the drug.

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Iron Toxicity Studies of Quelamycin

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SUMMARY

A previous phase I study demonstrated excessive generalized toxicity (20 of 21 patients) and cardiotoxicity (eight of 21 patients) of single-day intermittent quelamycin (NSC-267703) treatment, and a modified schedule was recommended to overcome this acute toxicity. In the present study, 40 mg/m² of quelamycin was administered iv on 2 or 3 consecutive days. This 2- or 3-day course was associated with a decrease in the incidence of general symptoms (five of nine patients) and a decrease in cardiotoxicity (none of nine patients). In addition, patients receiving multiple courses of quelamycin were evaluated. Clinical and pathologic findings supported the diagnosis of early hemochromatosis. In conclusion, quelamycin administration was associated with acute and chronic iron-overloading toxicity. Acute iron toxicity was prevented by the administration of quelamycin at a dose of 40 mg/m² iv on 3 consecutive days. On the other hand, hemochromatosis was an unexpected finding which requires further investigation before this drug is acceptable for broader studies.

[Cancer Treat Rep 63:909-913, 1979]

The results of a phase I clinical study of triferric Adriamycin (quelamycin) were recently reported (1). The study demonstrated that acute generalized toxicity occurred in almost all patients. In addition, immediate cardiotoxicity, characterized by tachycardia, arrhythmia, and electrocardiographic (ECG) changes, was observed in one third of the treated patients. Cardiotoxicity appeared to be unrelated to the quelamycin dose but correlated well with changes in the pretherapy ECG. Clinical findings suggested that iron toxicity from either excessive unbound iron in the drug preparation or from iron-chelated Adriamycin might have been responsible for some of the acute toxic effects. Consequently, it was postulated that a modification in the schedule could prevent some side effects, and further studies were designed. In addition, long-term studies were undertaken to investigate the iron-associated toxicity after multiple administrations of quelamycin. Young et al (2) have recently reported the presence of iron granules in lungs, liver, and kidneys of rodents treated with multiple doses of quelamycin. Based on these findings, selected patients from the phase I trial who received multiple courses of quelamycin underwent careful evaluation and the results are presented.

MATERIALS AND METHODS

Selected patients fulfilled the following eligibility criteria: (*a*) performance status (PS) of > 40% (Karnofsky scale); (*b*) pathologically proven non-small cell carcinoma of the lung or metastatic Adriamycin-resistant osseous and soft tissue sarcoma; (*c*) measurable disease; (*d*) major organ function within the normal limits; and (*e*) informed consent.

Quelamycin was supplied by Dr. M. Gosálvez (Department of Biochemistry, Clinica Puerta de Hierro, Madrid, Spain) in ampules containing a black lyophilized powder designed as a 25-mg equivalent of quelamycin to be reconstituted with 10 ml of water solvent. Quelamycin was administered slowly iv so that the infusion time was not < 15 minutes. After the infusion of quelamycin, the injection site was washed with a continuous iv running 5% dextrose in water solution over 2–3 hours.

Quelamycin was administered according to two different schedules. For the long-term toxicity analysis, patients from a previously reported phase I trial were studied. These patients received a single dose of quelamycin of 75–175 mg/m^2 every 3 weeks. In addition, a new schedule was investigated which consisted of 2 or 3 consecutive days of quelamycin administration at a daily dose of 40 mg/m^2.

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Prior to the initiation of treatment, a complete evaluation was performed which included medical history, physical examination, ECG, chest roentgenogram, urinalysis, cbc, liver profile tests, and creatinine clearance. Major evaluation tests were repeated at weekly intervals and included physical examination, tumor measurement, PS rating, blood cell counts, ECG, and serum chemistries. Patients receiving more than four courses of quelamycin underwent a second complete evaluation which included the baseline controls, electromyogram, electroencephalogram, and liver, bone, and skin biopsies, when clinically indicated.

Quelamycin was administered under very close medical observation. Patients with an abnormal baseline ECG were maintained in the intensive coronary unit 2 or 3 days after the administration of quelamycin. Changes in temperature, blood pressure, heart rate, and ECG were recorded and all side effects were clearly defined. Immediate acute toxicity was defined as all symptoms and alterations noticed during the first 48 hours of treatment.

Hematologic toxicity was defined according to the following criteria: *no toxicity*—leukocyte count of $\geqslant$ 4500/mm^3 and platelet count of $\geqslant$ 300,000/mm^3; *mild toxicity*—leukocyte count of 3000–4000/mm^3 and platelet count of 200,000–300,000/mm^3; *moderate toxicity*—leukocyte count of 2000–3000/mm^3 and platelet count of 100,000–200,000/mm^3; *severe toxicity*—leukocyte count of 1000–2000/mm^3 and platelet count of 50,000–100,000/mm^3; and *life-threatening toxicity*—leukocyte count of $\leqslant$ 1000/mm^3 and platelet count of $\leqslant$ 50,000/mm^3.

Hepatic, renal, cardiac, mucocutaneous, and neurologic toxic effects were also investigated.

Objective response was defined as a decrease by $\geqslant$ 50% in the size of a tumor as measured by the product of the two largest perpendicular diameters. Progression was defined as a $<$ 50% decrease in tumor size, mixed response, stable disease, and progressive tumor growth. Toxic death was defined as a death occurring during toxicity, including unexpected early death. Early death was defined as a death from progressive disease occurring before two cycles of treatment could be fully evaluated.

RESULTS

Analysis of Toxicity After Multiple Quelamycin Courses

Eight patients from a previously reported phase I study were evaluated. There were six male and two female patients ranging in age from 18 to 74 years (median, 58 years). Six patients had lung cancer: two epidermoid carcinoma and four anaplastic large cell carcinoma. One patient had metastatic osteogenic sarcoma and another had metastatic rhabdomyosarcoma. Four of these patients (three with lung cancer and one with osteosarcoma) showed an objective partial response to quelamycin treatment. Another two patients exhibited no change in the tumor size or general condition while receiving quelamycin. The other two patients in this report showed progressive disease after two or more treatments of quelamycin.

The dose of quelamycin given to these patients ranged from 75 to 175 mg/m^2 every 3 weeks. Patients receiving the highest dose of quelamycin were maintained at that level if myelotoxicity did not occur; otherwise, the quelamycin dose was reduced to the phase I recommended dose of 125 mg/m^2. At the time of this report the number of courses per patient ranged from two to eight and the total cumulative quelamycin dose per patient ranged from 250 to 1200 mg/m^2.

Acute toxicity after multiple courses of quelamycin was similar to that seen in the first course in four patients. Three of these patients refused to receive quelamycin on an outpatient basis. Another three patients stated that subsequent treatments caused fewer side effects. Acute toxicity consisted of early nausea and vomiting, shortness of breath, tachycardia and shakes, and delayed malaise and fever.

Four of the eight patients in this report belong to the group of patients who had acute cardiotoxicity after the first course of treatment: two patients had many atrial extrasystolic (AES) beats, one of them with a left anterior branch block, and the other two had atrial fibrillation (AF) which lasted 6 and 12 hours, respectively. After multiple courses of quelamycin, one of these patients continued to experience AES beats after each drug administration, and another patient had drug-induced AF after each course of therapy. The other two patients showed no further changes.

No cumulative hematologic toxicity was identified. In 29 fully evaluable observations in the first five cycles of treatment, the median leukocyte count nadir ranged from 2600 to 3900/mm^3 and the median platelet count nadir ranged from 175,000 to 265,000/mm^3.

Iron-Associated Toxicity

Three patients complained of malaise and mild abdominal pain, and a soft enlarged liver could be palpated 2–5 cm below the right costal margin. Liver biopsies were performed in six patients. The results obtained are shown in table 1. The general pathologic picture was that of a mild-to-moderate hemosiderosis with hepatocellular iron deposit. Only one patient showed discrete evidence of liver fibrosis. Hepatocellular alterations were characterized by mild fatty metamorphosis and signs of cell damage. The reticuloendothelial cells appeared to be normal and contained no iron deposits.

TABLE 1.—Liver biopsy after multiple quelamycin courses

Patient No.	Total quelamycin dose		Liver biopsy (score 0–4)			
	mg/m²	mg	Iron deposit	Hepatocellular damage	Fatty changes	Fibrosis
1	300	500	1	2	2	0
2	450	792+	3	0	0	0
3	475	800	2	1	2	0
4	625	1000+	1–2	0	0	0
5	750	1160+	2–3	1	2	0
6	875	1710+	3	2	2	1

Hyperpigmentation of the sun-exposed and unexposed areas of the skin was a common side effect. Four patients underwent punch biopsy of the skin which showed abnormal iron deposits with a mild increase in the activity of melanocytes. These changes were consistent with early hemochromatosis.

Bone marrow biopsies in two patients demonstrated a normal cell population and sideroblasts were considered to be within the normal limits.

Serum iron was elevated only during the first hours following quelamycin infusion and remained within the normal limits during the intervals between treatments. A rise in the serum iron capacity from 200 to > 300 μg/dl was noticed in three patients receiving multiple courses of quelamycin.

Liver function tests were considered normal for all patients receiving multiple courses of quelamycin except for one patient who had an abnormal sulfobromophthalein dye retention test; this patient had hepatic hemosiderosis and early liver fibrosis after a total quelamycin dose of 1200 mg/m².

One patient had chronic unexplained malaise, > 10% body weight loss, and significant weakness while maintaining a good partial response of her primary lung cancer. Quelamycin was discontinued and she returned to her normal condition after 1 month.

Finally, a dextrose tolerance test detected abnormal values in one patient. Hair growth was generally noticed after the second course of treatment. Secondary epilation was not observed during the course of therapy. Renal function, as measured by the creatinine clearance test, was unchanged. No neurologic toxicity was observed.

Analysis of the Schedule Modification (2 or 3 consecutive days of treatment)

Nine patients were studied: eight males and one female. Their ages ranged from 14 to 72 years (median, 58 years). Five patients had carcinoma of the lung: two epidermoid carcinoma and three large cell anaplastic carcinoma. Four patients had Adriamycin-resistant metastatic sarcoma; three had soft tissue sarcomas and one had bone sarcoma. Three patients had PSs of 60% and six patients had PSs of ⩾ 70% (Karnofsky scale). Four patients were considered to have an abnormal baseline ECG: two had many AES beats, one had supraventricular tachycardia, and one had a pre-excitation syndrome.

Six patients were initially treated with 40 mg/m² of quelamycin on 2 consecutive days and three patients were initially treated with 40 mg/m² of quelamycin on 3 consecutive days. The number of cycles per patient ranged from 1+ to 4+ at the time of this report (median number of cycles per patient, two).

The acute toxicity of the first course was generalized and manifested itself as nausea and vomiting, body aches, hypertension, bronchospasm, fever, and shakes occurring in approximately one half of the patients (table 2). It was usually noticed on the second or third day of treatment. Tachycardia or ECG changes were not observed except in one patient who had an increase in the frequency of AES beats after the drug infusion. Three patients had several serum iron determinations made daily at various intervals after treatment. Baseline serum iron ranged from 46 to 54 μg/dl. Serum iron peaks were found 1–3 hours after quelamycin infusion, and ranged from 184 to 254 μg/dl. Ten hours after treatment, serum iron levels approached the baseline level and 24 hours after quelamycin administration, serum iron levels were similar to baseline levels. No differences according to the day of treatment were observed. Two patients had total urinary iron determinations after quelamycin treatment; one patient excreted 24 mg of iron in the first 24 hours and the other patient excreted 31 mg of iron in the first 5 days.

Mild-to-moderate hematologic toxicity was noted in three patients and severe thrombocytopenia was observed in one.

DISCUSSION

This study confirmed the previously reported iron toxicity observed with quelamycin. Long-term quelamycin therapy was associated with pathologically proven changes consistent with iron-storage disease. The degree of iron deposition appeared to be related to the total amount of drug administered. The organ distribution, affecting the liver and the skin more than the bone marrow, suggested that iron deposition might follow the pattern of hemochromatosis.

Hemochromatosis is usually caused by an excessive iron overload from repeated blood transfusions or high iron intake. In certain cases hemochromatosis is a primary metabolic inherited disease. In the advanced stage the disease is characterized by liver cirrhosis, insulin-resistant diabetes, and skin pigmentation. However, in the early stages of the disease changes are subtle and generally unnoticed (3). Recent studies in high-risk populations (those with familial hemochromatosis and those on high iron-fortified diets) have demonstrated the early features of the disease (4,5). Widespread iron deposits were found in the reticuloendothelial system and in certain organs from the beginning of the disease. Differences could be observed in the iron-deposit prevalence according to the type of disease. In iron overloading, deposits were greater in bone marrow and Kupffer cells than in hepatic cells and a total iron storage of 30–50 g was needed before the clinical syndrome occurred (5). On the other hand, in primary hemochromatosis, iron preferentially deposited in liver cells, skin, and other organs with considerably less total body iron storage (4).

Two features were particularly interesting in this study regarding the pathologic iron storage. The first was what might be considered an excessive degree of changes relative to the total amount of iron administered. Total iron from distinct batches of quelamycin was measured and from the values obtained it was estimated that the total iron (free or unbound iron plus Adriamycin-chelated iron) given after multiple quelamycin courses was < 1 g/patient, which is roughly the iron equivalent of 3 or 4 units of whole blood transfusion. The second was that it was the pattern of iron deposition which appeared to be more consistent with primary hemochromatosis than with iron overloading. This is remarkable since, from the normal serum iron level and iron-binding capacity saturation, one could conclude that the studied patients had previously had a normal iron metabolism.

These two features might possibly suggest that quelamycin enhanced the intracellular iron deposition and acted by a selective transport of iron to certain tissues. If this is so, then hemochromatosis was a consequence of the metabolism of quelamycin instead of a side effect of the iron contained in the drug preparation.

Another observation of this study was that acute iron toxicity and immediate cardiotoxicity were no longer present when the drug was given in a 2- or 3-day schedule. The acute toxicity found when using the fractionated course was compared to the side effects found with the single-day course in table 2. As shown, the general symptoms and the cardiotoxicity which were prominent in patients receiving quelamycin at doses of 75–175 mg/m^2 in a single dose were mild or absent when the drug was given in a fractionated dose.

These results appeared to be consistent with a decrease in serum iron peak following quelamycin treatment. As shown in table 3, serum iron levels obtained in the 2- and 3-day course did not exceed what is considered to be the normal serum iron binding capacity. This might have explained the absence of clinical toxicity.

The first study did not show whether acute cardiotoxicity represented anthracycline cardiotoxicity, acute iron

TABLE 2.—Acute toxicity after the 1st course of quelamycin

		No. of patients with—							
		General toxicity				Cardiovascular toxicity			
	No. of patients	Aches	Nausea and vomiting	Fever	Shakes	Hypertension	Tachycardia (median frequency)	Abnormal ECG	Other clinical effects
Single dose of 75–125 mg/m^2									
Baseline normal ECG	6						110	—	—
Baseline abnormal ECG	3						130	3	—
Total	9	8	8	7	8	7			
Single dose of 150–175 mg/m^2									
Baseline normal ECG	5						120	—	—
Baseline abnormal ECG	7						145	5	4
Total	12	11	11	11	10	12			
2- or 3-day cycle of 80–120 mg/m^2									
Baseline normal ECG	5						105	—	—
Baseline abnormal ECG	4						105	1	—
Total	9	1	5	4	5	2			

TABLE 3.—Serum iron determinations after quelamycin treatment (median values of 4 different patients)

	Serum iron value (μg/dl) at—				
	Baseline	1 hr	3 hrs	6 hrs	10 hrs
Single-day course of 125 mg/m^2	50	307	220	170	70
2- and 3-day course of 80–120 mg/m^2	46	200	178	94	72

toxicity, or a combination of both. In the present schedule in this study, it appeared that high-risk patients, defined previously as those patients with an abnormal baseline ECG, had no significant toxic reactions. These results do not indicate whether iron or quelamycin was responsible for the toxic events, since the divided-dose schedule should have also produced a lower serum quelamycin concentration after each drug infusion.

Further studies are in progress to investigate the presence of cumulative cardiotoxicity after long-term quelamycin treatment.

In summary, quelamycin appeared to induce acute iron toxicity and chronic iron deposition with early hemochromatosis. Acute iron toxicity might be avoided by giving the total dose of quelamycin in a 3-day course. Improvement in the drug preparation to diminish the proportion of unbound iron and prevention of long-term iron deposition appear to be the relevant subjects for further studies.

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Preliminary Phase I Study of 4′-Epi-Adriamycin[1]

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SUMMARY

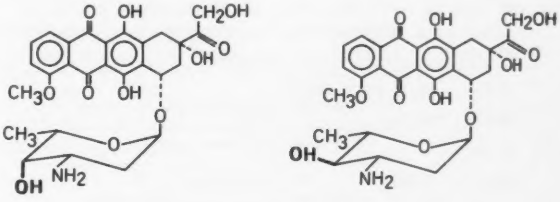
A phase I study of 4′-epi-Adriamycin (4′-epi-ADM) was performed in 22 patients with various types of advanced solid tumors. Very preliminary results would indicate that the drug produces a pattern of acute toxicity which is similar to that of Adriamycin. However, the incidence of vomiting, alopecia, and marrow suppression was less pronounced than that of Adriamycin. 4′-Epi-ADM prolonged the systolic time interval, although no patient presented clinical signs of cardiotoxicity. Two patients with renal carcinoma and malignant melanoma showed objective improvement. Present results suggest that further clinical studies with 4′-epi-ADM are indicated.

[Cancer Treat Rep 63:915–918, 1979]

4′-Epi-Adriamycin (4′-epi-ADM; fig 1) is one of the new Adriamycin (ADM) analogs which has exhibited interesting properties in experimental studies. 4′-Epi-ADM was found to interfere with nucleic acid synthesis and to inhibit mouse embryo fibroblast proliferation as does ADM (1). The drug also showed an antitumor activity equal to that of ADM against the following experimental tumors in mice: ascitic and solid sarcoma 180, Gross leukemia (2), L1210 and P388 ascitic leukemias, and transplanted C3H/He mammary carcinoma. Casazza et al (3) found that 4′-epi-ADM was slightly more effective than ADM against MSV-induced sarcoma, metastatic Lewis lung carcinoma, and MS-2 sarcoma.

Acute and subchronic toxicity studies in normal and tumor-bearing mice revealed that 4′-epi-ADM was less toxic than ADM, resulting in an increased therapeutic index (3). In rabbits treated iv for 3 consecutive days/week for a total of 6 weeks utilizing three dose levels, bone marrow suppression, gastrointestinal lesions, and myocardial damage were qualitatively similar after ADM and 4′-epi-ADM administration. A quantitative comparison showed that 4′-epi-ADM was about 25% less toxic, and in particular less cardiotoxic, than ADM.

Distribution studies (3) suggested that the decreased toxicity could be due to the different pharmacokinetic properties of the two compounds. The distribution of 4′-epi-ADM in kidneys, liver, heart, spleen, small intestine, and tumor tissue was similar to that of ADM. On the contrary, the concentration of 4′-epi-ADM was lower than that of ADM in both cardiac and splenic tissues at 1, 24, and 48 hours after administration. However, the drug concentration was equal to that of ADM in the tumor tissue at 1 and 6 hours after treatment. 4′-Epi-ADM was taken up to a greater extent than ADM in L1210 leukemia cells in vitro. The lower pKa of 4′-epi-ADM compared to ADM is another favorable property of this analog since compounds must be in their cationic form to be active and since the pH of the cytoplasm in tumor cells may be lower than it is in normal cells (4). The above-mentioned experimental data prompted us to undertake a phase I



ADRIAMYCIN

4′-EPI-ADRIAMYCIN

FIGURE 1.—Chemical structures of ADM and 4′-epi-ADM.

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clinical trial to verify the pattern of toxicity and pharmacokinetic properties of 4′-epi-ADM in cancer in man.

PATIENTS AND METHODS

Clinical studies with 4′-epi-ADM were started at the Istituto Nazionale Tumori in July 1977.

Drug Administration

4′-Epi-ADM was supplied by Farmitalia as a red powder in 10-mg vials. The molecular weight of 4′-epi-ADM is 579, which is identical to that of ADM. The drug was reconstituted with twice-distilled water and administered by direct rapid iv injection. Every course was given at 3-week intervals. The starting dose level was 10 mg/m^2. Progressive dose escalation was carried out according to the Fibonacci scheme up to a dose of 50 mg/m^2. Subsequently, we selected doses which would be comparable to therapeutically effective doses of ADM (75 and 90 mg/m^2). These doses were almost never escalated in individual patients who received multiple courses (table 1). Therapy was discontinued if there were definite signs of tumor progression or if the total dose of 4′-epi-ADM plus that of prior ADM was approaching 550 mg/m^2. The highest dose of 4′-epi-ADM which was administered in this study was 525 mg/m^2.

Patient Selection

All patients selected for phase I study had clinical evidence of disseminated malignancy which either had become resistant to conventional treatments or was known to be poorly responsive to current drugs (eg, chondrosarcoma, renal cancer). Only patients who had a life expectancy of > 2 months and a performance status of > 50 were eligible for the study. No patient had received radiotherapy and/or chemotherapy within 4 weeks prior to 4′-epi-ADM administration. Fourteen of 22 patients (64%) had previously been treated with chemotherapy. Four patients had previously received ADM (mean total dose, 207 mg/m^2; range, 120–320 mg/m^2). Patients were informed that 4′-epi-ADM was an experimental drug.

TABLE 1.—Dose of 4′-epi-ADM utilized during the preliminary phase I study

1st cycle	Escalation up to—	No. of additional cycles with initial dose	Prior ADM in mg/m^2*
10 (3)	20 (1), 50 (1), 90 (1)		320 (1)
20 (1)	30 (1)		150 (1)
30 (1)			
50 (4)	75 (2)	1 (1), 2 (1)	120 (1)
75 (11)	90 (1)	2 (3), 3 (4), 4 (1), 6 (1)	240 (1)
90 (2)		1 (1)	

*Nos. in parentheses = No. of patients.

Parameters of Evaluation

Pretreatment evaluation consisted of a complete medical history and physical examination with radiologic documentation of the extent of disease. Liver and bone scans were performed only when indicated by the clinical situation. Blood tests included complete hemogram, blood sugar, BUN, serum creatinine, uric acid, electrolytes, bilirubin, total proteins and albumin, alkaline phosphatase, and transaminases (SGOT and SGPT). All patients had an initial wbc count of $\geq 4000/\text{mm}^3$ and a platelet count of $\geq 110{,}000/\text{mm}^3$, as well as adequate liver and renal function tests (eg, bilirubin < 1.5 mg/100 ml and creatinine < 1.5 mg/100 ml). Hemograms were repeated at least once a week and blood chemistry tests were repeated prior to each course. Patients were followed closely for any evidence of toxicity and appropriate roentgenographic studies were repeated prior to every treatment course to document drug response.

Arterial blood pressure, pulse rate, electrocardiogram (ECG), and left ventricular systolic time intervals were recorded 30 minutes before each drug administration while patients were fasting and resting for about 20–30 minutes. The same parameters were repeated 1 hour after injection of 4′-epi-ADM. Systolic time intervals were determined according to the method of Weissler et al (5).

Pharmacokinetic Studies

Plasma levels and urinary and fecal drug recovery were determined in four patients after the administration of 50 (one patient) and 75 (three patients) mg/m^2 of 4′-epi-ADM. Sample extraction and chromatographic analysis were carried out according to the method of Bachur et al (6).

RESULTS

A total of 22 patients, 12 males and ten females with a median age of 50 years (range, 37–71 years), were entered in the study as of August 1, 1978. Their histopathologic diagnosis was as follows: malignant melanoma (five patients), renal carcinoma (four), squamous cell carcinoma of the head and neck (three), transitional cell carcinoma of the urinary bladder (two), thyroid carcinoma (two), chondrosarcoma (two), bronchogenic carcinoma (one), liposarcoma (one), ovarian carcinoma (one), and adenocarcinoma of unknown origin (one).

TABLE 2.—% of patients with toxic effects after the 1st cycle of 4′-epi-ADM

Toxicity	% of patients receiving doses (mg/m^2) of— 50 (total = 4 patients)	75 (total = 11 patients)	90 (total = 2 patients)
Nausea	50	36	50
Vomiting	25		
Stomatitis		9	
Diarrhea			50
Fever*		18	
Hair loss	25	64	50
Leukopenia†	50	45	50
Thrombocytopenia‡	25	9	
Renal toxicity	0	0	0
Liver toxicity	0	0	0
Phlebitis	0	0	0

*38°C.
†Wbc count < 4000/mm^3.
‡Platelet count < 110,000/mm^3.

TABLE 3.—Hair loss after various cumulative doses of 4′-epi-ADM

	No. of patients with toxic effects/No. treated with total doses (mg/m^2) of—			
Hair loss	10–100	101–200	201–300	301–525
Partial	10/22 (45%)	4/16 (25%)	3/11 (27%)	1/4 (25%)
Complete	1/22 (4.5%)	7/16 (44%)	5/11 (45%)	2/4 (50%)

TABLE 4.—ECG changes in 22 patients after 4′-epi-ADM

ECG changes	No. of patients
Arrhythmias	
Coronary sinus rhythm	1
Atrial premature beats	3
Ventricular premature beats	2
ST-T changes	
RS-T depression	1
Flattening of T wave	4
Combination of ECG changes	3

Acute Toxicity

All patients and treatment courses were considered evaluable for acute toxic effects. No toxic effects were detected after single doses of 10, 20, and 30 mg/m^2. The toxic manifestations observed after one cycle with 50, 75, and 90 mg/m^2 are shown in table 2. Although the number of patients is still limited, some side effects such as vomiting, hair loss, and myelosuppression, which almost invariably occur after comparable single doses of ADM, appear to be less frequent after 4′-epi-ADM. Loss of hair did not occur in all patients even after high total doses (table 3). Leukopenia was not clearly dose-related. Thrombocytopenia occurred less frequently than leukopenia and a platelet count of < 50,000/mm^3 was found in only one patient after the first dose of 50 mg/m^2. In patients given 50 and 75 mg/m^2, the median wbc count nadir occurred on about the 11th or 12th day. In patients receiving 75 mg/m^2, the median platelet count nadir occurred on the eighth day. All patients recovered from myelosuppression before the end of the third week. A mild degree of anemia developed in most patients treated at doses of $\geqslant$ 50 mg/m^2. However, at this point, drug-induced anemia cannot be differentiated from disease-induced anemia.

Table 4 summarizes the ECG changes observed in eight patients after different intervals from the first dose of 4′-epi-ADM. As already shown with ADM, flattening of T waves was the most common abnormality observed. This change occurred at doses of > 30 mg/m^2 and was reversible upon drug discontinuation. The variations of systolic time interval (PEP/LVET) after various cumulative doses are shown in table 5; as shown, the systolic time interval increased over the basal value. While there was no difference between 100- and 200-mg/m^2 doses, patients given a total dose of > 200 mg/m^2 showed a considerable increase in the PEP/LVET ratio. The systolic time interval of the patient given a dose of 525 mg/m^2 was 0.446. No patient developed cardiomegaly or clinical signs of heart failure.

Therapeutic Response

Objective tumor response was observed in two patients. The first patient, who had clear cell carcinoma of the kidney which was resistant to medroxyprogesterone

TABLE 5.—Variations of systolic time interval (PEP/LVET) after various cumulative doses of 4′-epi-ADM

	Basal value (11 patients)	PEP/LVET ratios in patients receiving total doses (mg/m^2) of— 100 (11 patients)	200 (6 patients)	300 (4 patients)
Median value	0.326	0.350	0.350	0.394
Upper value	0.375	0.451	0.412	0.460
Lower value	0.307	0.295	0.276	0.368

acetate, hydroxyurea, and cyclophosphamide, received 4′-epi-ADM at a dose of 75 mg/m^2. A complete remission of recurrent neoplastic pleural pulmonary effusion occurred while pulmonary metastases remained essentially unchanged. Response occurred after the first cycle, and this remission has persisted for > 6 months despite a discontinuation of treatment at a total dose of 525 mg/m^2. The second patient, who had malignant melanoma with bilateral lung metastases, received three cycles of 4′-epi-ADM at a dose of 75 mg/m^2. A transient improvement was observed on two successive chest roentgenograms after the first cycle.

Pharmacokinetic Studies

Preliminary data obtained from examination of blood and urine samples in four patients treated with 50 and 75 mg/m^2 suggest that 4′-epi-ADM has a pharmacokinetic behavior similar to that of ADM. As previously observed in patients treated with ADM (7), three distinct portions of the plasma concentration curve were recognized with 4′-epi-ADM. Drug concentrations in plasma (total fluorescence) were 3.5–8.0 μg/ml at 2 minutes, about 0.1 μg/ml at 24 hours, and about 0.03 μg/ml at 172 hours. The half-life was 4 minutes in the fast distribution phase and 2.4 hours in the slow distribution phase. In the long elimination phase, the half-life varied from 3 to > 100 hours. The urinary excretion rate reached a mean value of 15% in 7 days. The examination of thin-layer chromatographic plates showed that 4′-epi-ADM was extensively metabolized. Its metabolites appeared very rapidly in the blood where they could be detected at our earliest sampling times. A large portion of the urinary fluorescence is accounted for by some as yet unidentified metabolites. No unchanged 4′-epi-ADM was detected in stool extracts.

DISCUSSION

4′-Epi-ADM is the first ADM analog to be tested in human cancer at the Istituto Nazionale Tumori. The toxicologic as well as the clinicopharmacologic results reported in this paper are very preliminary. However, at doses similar to the therapeutic doses of ADM, 4′-epi-ADM produced less acute toxicity. In fact, pronounced, and often protracted, nausea and vomiting occurs almost invariably when single doses of ADM of > 50 mg/m^2 are administered and alopecia and myelosuppression are seen consistently after single doses of > 75 mg/m^2. With ADM, platelet depression is usually less frequent than leukocyte depression (8), and the present findings with 4′-epi-ADM showed that both the incidence and degree of thrombocytopenia were moderate-to-low, even in patients previously treated with chemotherapy and radiotherapy. Available data from pharmacokinetic studies fail to provide an explanation for the difference in toxicology between ADM and 4′-epi-ADM. Preliminary results of marrow toxicity should be confirmed in a larger series of previously untreated patients.

Preliminary cardiologic studies indicate that 4′-epi-ADM produces an increased systolic time interval. It is well known that endomyocardial biopsy is a reliable method for monitoring cardiac damage due to anthracyclines in man (9), and this type of invasive method should also be applied to better assess 4′-epi-ADM cardiotoxic effects. More recently, Merski et al (10) have documented ultrastructural changes of cardiac and skeletal muscle cells in rats treated with ADM. These changes included nucleolar segregation and altered distribution of the perinuclear chromatin. The similarities in response of skeletal and cardiac muscle suggest that ultrastructural analysis of skeletal muscle biopsies may be useful in evaluating ADM cardiotoxicity. We first plan to confirm Merski's results in animals and then try to assess the predictive value of muscle biopsy in patients subjected to treatment with various dose schedules of ADM and 4′-epi-ADM. If it is confirmed that skeletal biopsy is useful in predicting the presence of ADM-induced myocardial damage, the technique would be safer and probably more accurate, since peripheral biopsies are easier to repeat than endomyocardial samples. The technique would also facilitate the screening of various ADM analogs which could be candidates for clinical use on the basis of their decreased cardiotoxicity compared to ADM.

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Initial Clinical Evaluation of *N*-Trifluoroacetyladriamycin-14-valerate (AD-32), an Adriamycin Analog[1]

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SUMMARY

***N*-Trifluoroacetyladriamycin-14-valerate (AD-32) is superior to Adriamycin in murine L1210 and P388 leukemias and in a number of solid tumor systems, including Ridgway osteogenic sarcoma and Lewis lung carcinoma. In preclinical toxicology studies, AD-32 was less toxic than Adriamycin in both tumor- and non-tumor-bearing mice and in rabbits. An initial clinical trial was carried out in 23 patients who received a total of 74 courses of AD-32 over a dose range of 100–700 mg/m^2 administered at 21-day intervals. Hydrocortisone given during the period of infusion prevented all clinical manifestations of acute toxicity. The AD-32 dose-limiting toxicity, leukopenia, was comparable to that of Adriamycin at a dose of 10:1, but at these equivalently myelosuppressive doses, AD-32 induced less gastrointestinal toxicity and alopecia than Adriamycin and it did not cause local tissue damage following inadvertent paravenous extravasation. Although two responses are reported, the therapeutic activity of AD-32 cannot be assessed because of an inadequate number of patients in any given tumor type. A phase II study is being initiated at a dose of 600 mg/m^2 given at 21-day intervals.**

[Cancer Treat Rep 63:919–923, 1979]

The anthracycline antitumor antibiotics are established chemotherapeutic agents for the treatment of a wide spectrum of human malignancies, including both solid tumors and leukemias. The use of these drugs is limited by both acute toxicity (myelosuppression) and chronic, dose-related cardiac toxicity. In connection with a program to develop anthracycline analogs with greater therapeutic activity and/or less toxicity, Israel et al (1) first prepared *N*-trifluoroacetyladriamycin-14-valerate (AD-32) in late 1973. This compound, a semisynthetic analog of the antibiotic Adriamycin, differs from the parent compound in having a five-carbon straight-chain ester function at the 14-carbinol position and a trifluoroacetyl substituent on the amino group of the glycoside (fig 1).

AD-32 has undergone comparative evaluation with Adriamycin in murine tumor model systems. When tested against the L1210 and P388 leukemia models, AD-32 was superior to Adriamycin, both in terms of the percent increase in lifespan and the percentage of animals achieving long-term survival (1,2). These results have been confirmed in independent studies in other laboratories (3). Additional studies established that the therapeutic superiority of AD-32 over Adriamycin in the murine systems was not due to differences in the formulation vehicles originally used (2,3). AD-32 was also superior to Adriamycin when it was tested against a P388 subline selected for relative Adriamycin resistance. Significant antitumor activity was also seen with AD-32 against a number of murine solid tumors, eg, Ridgway osteogenic sarcoma (2) and Lewis lung carcinoma (3).

The mechanism of antitumor action of AD-32 is under investigation. In contrast to Adriamycin, AD-32 does not bind to double helical DNA (4). In vitro AD-32 rapidly

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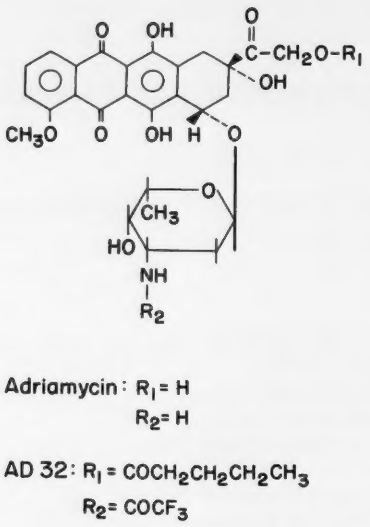

FIGURE 1.—Structures of AD-32 and Adriamycin.

enters cells and shows cytofluorescence localized within the cytoplasm; Adriamycin uptake is slower, with cytofluorescence seen only in the nucleus (5).

AD-32, when compared to Adriamycin, is less toxic in both tumor- and non-tumor-bearing mice and in rabbits. In non-tumor-bearing mice, no deaths were seen with doses of AD-32 at 15 times the 100% lethal single dose of Adriamycin (20 mg/kg) (2). Similarly, no toxic deaths were seen with four daily doses of 20–90 mg/kg of AD-32, a dose range which includes the optimal dose level of AD-32 in in vivo antitumor assays. In L1210 leukemia-bearing mice, an AD-32/Adriamycin toxic dose ratio of 12 was derived from pooled data from multiple experiments (2).

Additional studies were done in rabbits, a model that can be used to evaluate both toxic dose levels and potential anthracycline-induced cardiomyopathy (6). In rabbits, single doses of Adriamycin (2 mg/kg) and AD-32 (30 mg/kg) were found to produce equivalent levels of myelosuppression. Weekly administration of AD-32 to rabbits, at doses 15 times those of Adriamycin on a weight basis and for longer periods of time, resulted in significantly less weight loss, alopecia, local tissue necrosis, and cardiac toxicity compared to rabbits similarly treated with Adriamycin (7). Myocardial damage was assessed according to the histologic scoring system developed at the Stanford University Medical School, Calif (8).

PHARMACOLOGY

Pharmacology studies with mice (9), rats (10), and monkeys (11) have shown that, in contrast to Adriamycin, AD-32 is extensively metabolized, and its biotransformation products are rapidly eliminated. The major products seen in biologic fluids (serum, bile, and urine) are *N*-trifluoroacetyladriamycin and *N*-trifluoroacetyladriamycinol. Conjugates of *N*-trifluoroacetyladriamycin and *N*-trifluoroacetyladriamycinol are also seen in bile and urine. Adriamycin, although seen occasionally in small amounts in some samples, is not a significant metabolite of AD-32, thus suggesting that AD-32 is not serving as an Adriamycin prodrug. A preliminary analysis of serum and urine samples from patients treated with AD-32 in connection with phase I clinical studies indicates that the pharmacology of AD-32 in man is similar to that seen in laboratory animals (12). A more detailed analysis of AD-32 clinical pharmacology will be the subject of a subsequent paper.

PATIENT SELECTION

All patients had histologic confirmation of a malignant disease which had become refractory to other therapies or for which there was no therapy of proven clinical benefit. All patients were ambulatory, had an estimated survival of $>$ 2 months, and had normal hematologic, renal, and hepatic functions. Objective measurable disease was not required. Patients previously treated with other anthracyclines or with a history of left ventricular dysfunction were excluded. Written informed consent was obtained from all patients prior to treatment.

TREATMENT PLAN

Bulk AD-32 was manufactured by Farmitalia, Milan, Italy, under an agreement involving the Sidney Farber Cancer Institute, Adria Laboratories Inc, Columbus, Ohio, and the National Cancer Institute. The material agreed in all physical, chemical, and biologic respects with samples of AD-32 originally prepared by Israel et al (1,13). The drug was distributed into vials, each containing 200 mg of lyophilized AD-32.

In contrast to Adriamycin, AD-32 is relatively water-insoluble, and therefore must be solubilized in a lipophilic solvent medium. The reconstitution for clinical use of the 200-mg vials uses equal volumes of polyethoxylated vegetable oil (Emulphor EL620) and absolute ethanol to a total volume of 5.7 ml. This solution, containing 35 mg of AD-32/ml, is then diluted to nine times its volume with 0.9% saline without preservative. This solution is again diluted 1:10 with 5% dextrose or normal saline to achieve a final concentration of 0.35 mg of AD-32/ml of 0.5% Emulphor-0.5% ethanol-99% aqueous vehicle. Material thus prepared shows no particulate matter under microscopic examination and passes freely through a 0.45-μ membrane filter. When protected from light the preparation is physically and chemically stable at room temperature for at least 48 hours. The preparation was given as a continuous 24-hour infusion using an IMED pump (IMED Corp, San Diego, Calif). Three patients in the early stages of the trial received a formulation containing 3.5 mg of AD-32/ml of 5% Emulphor-5% ethanol-90% saline. Based upon localized toxicity (vide infra), subsequent patients received the more dilute formulation described above.

A safe phase I starting dose for most drugs can be extrapolated by using 0.1 of the maximally tolerated dose determined in preclinical systems (14). The AD-32 dose was extrapolated from both preclinical toxicology studies in mice and rabbits and from the maximally tolerated dose of Adriamycin in man. The maximally tolerated dose of AD-32 in preclinical toxicology studies is 600–900 mg/m^2 (7) or approximately 10–15 times the equivalently myelosuppressive dose of Adriamycin. Thus, a 100-mg/m^2 starting dose was consistent with both the AD-32 preclinical data and the factor of 0.1 of an equitoxic maximally tolerated dose of Adriamycin (7,15).

A 5% Emulphor solvent system has been associated with an acute pain syndrome when used with parenteral methyl-CCNU at doses of > 3.6 ml/m^2/30 minutes (16). For AD-32, a 24-hour continuous infusion was chosen, based on the assumption that the Emulphor vehicle toxicity was dose-rate-dependent.

A 21-day treatment interval was extrapolated based upon previous experience with Adriamycin schedules. After three patients were evaluable for toxicity at 100 mg/m^2, two patients were entered at 200 mg/m^2 (table 1). At the next dose level (400 mg/m^2) toxicity was encountered. Subsequent dose escalations were carried out to 500, 600, and 700 mg/m^2. New patients were entered at the highest tolerable evaluated dose. Doses were escalated within patients, since no cumulative toxicity was documented in preclinical toxicology studies. Dose adjustments were based on the toxicity of the previous course. Patients were taken off the study when the maximally tolerated dose had been encountered and/or objective tumor progression had occurred.

Study Parameters

Patients had routine history and physical examination, with biochemical and radiographic documentation of the extent of disease. Blood was obtained prior to treatment and at twice weekly intervals during treatment for cbcs, and at weekly intervals for BUN, creatinine, LDH, transaminases, alkaline phosphatase, total protein, albumin, and bilirubin. In order to evaluate vehicle toxicity, arterial blood gas determinations were obtained prior to and upon completion of the AD-32 infusion.

TABLE 1.—AD-32: phase I dose escalations

Dose (mg/m^2)	No. of patients (initial treatment)	No. of courses
100	3	3
200	2	5
400	9	25
500	7	14
600	2	15
700	0	12
Totals	23	74

RESULTS

Over an 8-month period, 23 patients (11 males and 12 females) with metastatic solid tumors were entered in the study. Their median age was 56 years (range, 26–68). Ten patients had been treated and had failed prior chemotherapy and six had received radiation therapy. Nine had received no prior systemic antineoplastic therapy.

Toxicity

No significant toxicity occurred at doses of < 400 mg/m^2. At 400 mg/m^2, five of eight courses resulted in an acute systemic reaction, presumably related to the Emulphor vehicle. Within 12–16 hours of initiating the infusion, these five patients developed chest pain, shaking chills, fevers (101°F–103°F), hypotension, and diffuse bronchospasm. Arterial oxygen concentrations and chest roentgenograms showed no changes suggestive of pulmonary emboli. However, iv hydrocortisone was administered to all patients on the rationale that this reaction was consistent with fat embolization (17–19). The entire complex resolved within 2–4 hours after stopping the AD-32 infusion and initiating the hydrocortisone. In all subsequent courses of AD-32, 100 mg of hydrocortisone was given iv just prior to the infusion (0 hour), and at 8, 16, and 24 hours during the infusion. This maneuver reduced the overall incidence of acute reactions to three of 53 (6%) courses at a dose of $\geqslant 400$ mg/m^2. These three toxic courses were much less severe and did not necessitate discontinuing the AD-32 infusion.

Perfusion lung scans were performed before and after AD-32 infusion in eight patients. One patient developed new segmental defects on a perfusion lung scan performed after an acute hypoxic episode 12 hours after completion of an AD-32 infusion. An autopsy done the following day was consistent with the presence of necrotic tumor in the alveolar spaces. The other seven patients had no significant changes in their post-treatment lung scans. Pre- and post-treatment arterial blood gases showed no significant changes in arterial oxygen concentrations, but insignificant changes in arterial-alveolar oxygen gradients were noted.

Severe phlebitis, attributable to a slow infusion rate, was encountered in the first patient treated at a dose of 200 mg/m^2 with the more concentrated solution containing 3.5 mg of AD-32/ml. However, with dilution to the 0.35-mg/ml solution, which is now used, no significant phlebitis

TABLE 2.—AD-32 myelosuppression

	Dose (mg/m^2)			
	400	500	600	700
No. of patients	13	10	8	4
No. of evaluable courses	21	13	13	11
Wbc count ($\times 10^3/mm^3$)				
Median	3.2	2.2	1.8	1.3
Mean	3.3	2.4	2.2	1.6
Range	1.5–6.4	1.4–4.3	1.2–4.3	0.1–3.5
Platelet count ($\times 10^3/mm^3$)				
Median	230	211	221	94
Mean	239	208	225	109
Range	104–440	58–381	138–412	22–248

TABLE 3.—Therapeutic evaluation of AD-32

	No. of—		
Tumor	Patients entered	Patients evaluable	Responses
Lung (non-oat-cell)	5	3	1
Bladder	3	2	1
Colon	3	3	0
Melanoma	2	2	0
Sarcoma	3	1	0
Unknown primary	3	2	0
Other	4	1	0
Totals	23	14	2

was observed in any subsequent course. In fact, with this more dilute solution, significant subcutaneous damage has not been observed at any dose of AD-32.

Drug-induced myelosuppression was dose-related (table 2). Leukopenia was dose-limiting at doses of ≥ 600 mg/m^2. The median wbc count at 600 mg/m^2 was 1.8×10^3/mm^3, with a mean of 2.2×10^3/mm^3 and a range of 1.2–4.3×10^3/mm^3. The median nadir day for leukopenia was Day 14 with a recovery always occurring by Day 21. Thrombocytopenia was not as pronounced. No cumulative toxicity was noted. Based on these data, 600 mg/m^2 is an appropriate starting dose for previously untreated patients with adequate bone marrow reserve.

Gastrointestinal toxicity was minimal. Nausea occurred in only four of the 23 patients treated at a dose of ≥ 400 mg/m^2. AD-32 induced some, but not complete, alopecia. No mucositis or renal or hepatic toxicity has been observed.

At this preliminary time, cardiac toxicity, as manifested by auscultatory findings and/or symptomatic congestive heart failure, has not been encountered. However, only three patients are at risk for cardiomyopathy, having received doses of > 3 g/m^2. Histologic interpretation of a perimortem endomyocardial biopsy is pending in one patient who had a cumulative dose of 3.2 g/m^2 of AD-32.

Response

Fourteen of the 23 patients evaluable for toxicity were evaluable for response. Nine patients cannot be evaluated for response: five patients have received only one course and are still in the study, two patients had no measurable disease, one patient was lost to followup after one course, and one patient had therapy interrupted because of fever unrelated to the drug. Histologic cell types of the 14 evaluable patients are shown in table 3.

A patient with transitional cell carcinoma of the bladder showed marked symptomatic improvement and objective chest roentgenogram resolution of lymphangitic carcinomatosis. A patient with adenocarcinoma of the lung had a mixed response with an objective decrease in a hilar mass but persistence of a pulmonary nodule. No other objective responses have been observed.

DISCUSSION

The toxicity of AD-32 has been described in 23 patients who received 74 courses of treatment. Both vehicle and drug toxicity have been encountered. The Emulphor vehicle system, used to solubilize the lipophilic AD-32, is associated with an acute pain syndrome and may potentially cause chronic toxicity. The pathophysiology of this adverse effect is not well-understood. The cardiorespiratory changes may be similar to experimental observations (20). In traumatic fat embolization, fat particles are trapped in the pulmonary vasculature (19). If fat embolization is invoked as the pathogenic mechanism, then the formation of fat particles must be occurring in vivo, since the Emulphor-alcohol-AD-32 formulation, as administered, does not contain particulate matter. With concurrent steroids, patients are asymptomatic, but may show a minimal subclinical increase in their arterial-alveolar oxygen gradient. This change may represent a ventilation and/or perfusion alteration. Additional pulmonary physiologic studies will be performed as part of the phase II study.

The dose-rate of Emulphor at an AD-32 dose of 400 mg/m^2 was 3% of that used in the methyl-CCNU phase I study (16), but the total amount of Emulphor given was double. This calculation would suggest that the acute toxicity is dose-, rather than dose-rate-, dependent. Additional dose schedules and shorter infusion rates are under consideration.

As with Adriamycin, leukopenia is the acute dose-limiting toxicity of AD-32. AD-32 given at ten times the commonly used dose of Adriamycin results in comparable myelosuppression. At these equivalently myelosuppressive doses, AD-32 gastrointestinal toxicity was minimal compared to that produced by Adriamycin. Also, in contrast to Adriamycin, inadvertent paravenous extravasation of AD-32 caused no significant local tissue damage. The degree of alopecia with AD-32 was also less than with Adriamycin.

The therapeutic activity of AD-32 cannot be assessed in this phase I study because of inadequate numbers of patients. Fourteen patients with ten solid tumor cell types were evaluable for response. Antineoplastic activity of AD-32 was demonstrated in two patients. Eight of 14 patients had stable disease while receiving treatment, but six of 14 patients had objective progression. Ten of the 23 patients remain in the study from 1 to 7 months.

A phase II activity-seeking study is being initiated at a dose of 600 mg/m^2 given at 21-day intervals. Previously untreated patients with soft tissue sarcomas, bladder cancer, and other Adriamycin-sensitive tumors will be entered to determine the activity and to evaluate the chronic toxicity of AD-32. Patients with bronchogenic carcinoma (non-oat-cell), colon cancer, melanoma, and other Adriamycin-resistant tumors will also be entered in order to evaluate the broader spectrum of AD-32 antitumor activity demonstrated in animal systems. Definitive comparisons of AD-32 and Adriamycin with respect to both therapeutic activity and cardiac toxicity will depend on comparative prospective trials.

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Clinical Studies With Rubidazone[1]

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SUMMARY

One hundred and thirty-three patients, 94 with acute leukemia and 39 with solid tumors, received rubidazone, alone or in combination, at M. D. Anderson Hospital. The initial study, a phase I–II study carried out in 39 patients with acute leukemia, revealed substantial antileukemic activity with optimal results at a dose level of 450 mg/m^2. Toxic manifestations included an acute reaction suggestive of histamine release with dose-limiting mucositis at a dose of 600 mg/m^2. Forty-seven patients with acute leukemia were treated at phase II dose levels. Thirteen of 32 patients (42%) with acute myelogenous leukemia and seven of ten patients (70%) with acute lymphocytic leukemia achieved complete remission. Twenty-seven previously untreated patients with acute leukemia who were $>$ 50 years old were treated with rubidazone in combination with cytosine arabinoside, vincristine, and prednisone. Fifteen patients (56%) achieved complete remission including 12 of 15 patients (73%) who were treated at a dose of 200 mg/m^2 of rubidazone on Day 1 and a dose of 70 mg/m^2/day × 7 days of cytosine arabinoside (continuous infusion). For patients with solid tumors, the dose-limiting toxic effect was myelosuppression at a dose of 200 mg/m^2. Other toxicity at that dose level was minimal. The best responses were seen in patients with carcinoma of the parotid with two of four evaluable patients showing objective tumor regression. Of six previously untreated patients with thyroid carcinoma none responded, and in a phase II study of patients with breast cancer there were no partial remissions among 13 patients. Cardiac toxicity, manifested by congestive heart failure, occurred in seven patients at cumulative doses of 1050–2600 mg/m^2 of rubidazone; all patients had had prior anthracycline therapy at low doses. Rubidazone has been shown to be an active antileukemic agent, but appears to be less active than Adriamycin in our studies of patients with solid tumors.

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Rubidazone is superior to daunorubicin in several animal tumor systems (1). On this basis, it was introduced into clinical trials in patients with acute leukemia by Bernard and his associates (2). These studies were extended to 170 patients by Jacquillat et al (3). A complete remission (CR) rate of 57% was obtained in 70 previously untreated patients with acute myelogenous leukemia (AML); slightly lower rates were obtained in previously treated patients. The investigators felt that the drug had a higher therapeutic index than daunorubicin, was easier to use, and was less likely to produce irreversible bone marrow aplasia.

Based on their encouraging reports, we instituted a phase I–II study of rubidazone in previously treated patients with acute leukemia, but chose a single dose in our studies because of the long plasma half-life of the anthracyclines (4,5).

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MATERIALS AND METHODS

Phase I study: acute leukemia.—Thirty-nine adults with acute leukemia were entered in our phase I study (6). Thirty-two patients had AML, six had acute lymphocytic leukemia (ALL) or acute undifferentiated leukemia (AUL), and one had chronic myelogenous leukemia in blastic crisis (CML-BC). All patients had been treated with previous chemotherapeutic regimens and 29 had received prior anthracycline therapy consisting of Adriamycin or daunorubicin (one patient) at doses ranging from 40 to 270 mg/m^2 (median, 165 mg/m^2). Rubidazone was initially supplied for our studies by the Societé des Usines Chimiques, Rhône-Poulenc, Paris, France, through Rhodia, Inc, New York, NY. Later, the drug was obtained through the Investigational Drug Branch, Cancer Therapy Evaluation Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, Md. The first three patients were treated at a dose of 450 mg/m^2. Because of the French experience with considerably higher doses (750 mg/m^2 $\times$ 5 days), the next ten patients received 600 mg/m^2 or an equivalent dose based on plasma levels in patients with abnormal hepatic and/or renal function. Because acute toxicity occurred at this dose level, the dose was divided, 300 mg/m^2 on Days 1 and 5 for the next ten patients, and subsequent patients were treated at single doses of 450 or 300 mg/m^2. The drug was reconstituted to a concentration of 12.5 mg/ml in a glycine buffer, pH 7.6, added to 5% dextrose in water to a final concentration of 1–2 mg/ml, and infused at 500 ml/hour. Patients received premedication with 50 mg of Benadryl iv to prevent acute toxic reactions. If there was no reduction of leukemic infiltrate 2 weeks after the first dose, a second dose was given, occasionally at an escalated level. Platelets, granulocytes, and antibiotics were administered when indicated. Hemograms and tests of renal and hepatic function were monitored frequently, and electrocardiograms (ECGs) were obtained before each rubidazone dose and were evaluated according to the method of Minow et al (7). After CR was achieved, patients received consolidation therapy with one dose of 300 mg/m^2 (occasionally 200 mg/m^2) and two further doses adjusted to provide acceptable myelosuppression (lowest blood cell counts > 500 granulocytes/μl and $> 50{,}000$ platelets/μl). The usual maintenance dose was 150–200 mg/m^2 but varied from 150 to 375 mg/m^2. Thereafter, some patients received continued rubidazone and other patients received alternative regimens adjusted according to prior therapy.

Phase II study: acute leukemia.—An additional 28 previously treated adults with acute leukemia were entered in our study at the optimal doses from the phase I study (300–459 mg/m^2). Seventeen patients had AML, six had ALL or AUL, and five had CML-BC. Data from these patients were analyzed together with data from patients treated in the phase I study at the same doses.

Phase II combination study: acute leukemia.—Twenty-seven previously untreated patients with acute leukemia who were > 50 years old were treated with the ROAP protocol (rubidazone, vincristine, cytosine arabinoside [ara-C], and prednisone) (8). A high-dose regimen, consisting of 200 mg/m^2 of rubidazone, 2 mg of vincristine, 70 mg/m^2/day $\times$ 7 by continuous infusion of ara-C, and 100 mg/day of prednisone orally, was administered to 15 patients, while the remaining 12 received a low-dose regimen with 20% less rubidazone and ara-C.

Phase I study: solid tumors.—Based on myelosuppressive toxicity which occurred during remission in the patients with acute leukemia, a dose of 200 mg/m^2 was considered to be appropriate for patients with solid tumors (9). Twenty-six patients were entered in the study but data were available for only 21. Most patients were previously treated; however, seven with thyroid carcinoma, four of five with parotid carcinoma, one of two with hepatoma, and one with pancreatic carcinoma were previously untreated. Other patients had adenocarcinoma of the lung (two), breast (one), or prostate (one); small-cell carcinoma of the lung (two); squamous cell carcinoma of the bladder (one); and unclassified carcinoma. Seventy courses of therapy were administered to 21 patients. All patients received at least two courses, ten received three, and two received seven. Doses varied from 75 to 400 mg/m^2 and were adjusted to produce acceptable myelosuppression as previously noted in leukemia maintenance therapy.

Phase II study: breast cancer.—Thirteen patients previously treated with cyclophosphamide, methotrexate, and 5-fluorouracil were treated with 150 mg/m^2 of rubidazone every 3 weeks. The low dose was used because of the poor tolerance of these patients to myelosuppressive chemotherapy. All patients were postmenopausal, and most had received endocrine therapy.

RESULTS

Phase I Study: Acute Leukemia

Response

Of the 20 patients treated at the high-dose levels (600 mg/m^2 in one dose or 300 mg/m^2 on Days 1 and 5), only three (15%) achieved CR; one had CML-BC and the other two had AML. All three responders were female. At the low dose levels, ten of 19 patients (53%) achieved CR; they will be included in the analysis of phase II results.

Toxicity

The toxic manifestations of rubidazone (6) were similar to those expected from an anthracycline antibiotic and most resembled those observed with single high-dose daunorubicin (10). Acute reactions in our first 12 patients (31%) consisted of fever and chills in nine, mild to gen-

TABLE 1.—Major dose-related toxic effects of rubidazone in acute leukemia

Toxic effect	No. of patients (%) Low-dose	No. of patients (%) High-dose	*P* value
No. of patients treated	19	20	
Mucositis	5 (26)	14 (70)	0.01
Severe	0	9 (45)	0.001
Renal	2 (11)	5 (25)	0.23
Hepatic	5 (26)	13 (72)	0.02
Severe	0	3 (17)	0.12
Persistent marrow hypoplasia	0	2 (10)	0.26
Infection	12 (63)	19 (95)	0.02
Bleeding	2 (11)	4 (20)	0.36
Drug-related death during induction	1 (5)	11 (55)	0.001

eralized urticaria in eight, wheezing in two, flushing in two, and postural hypotension in two. These symptoms occurred with the first dose of rubidazone, even in patients without prior exposure to anthracycline antibiotics. They were ameliorated by iv administration of an antihistamine and did not get worse with subsequent doses. These acute reactions have been completely prevented in subsequent patients by iv antihistamine premedication. We doubt, therefore, that these reactions represent true drug allergy and suspect direct drug-related histamine release. Minor toxic reactions included alopecia in all patients, nausea and vomiting in 35 (90%), phlebitis in seven (18%), distant pain in four (10%), rash in three (8%), and cellulitis in one (3%).

The major subacute toxic reactions were definitely dose-related. The clearest dose-response curve was noted for mucositis which was tolerable at 450 mg/m^2 but not at 600 mg/m^2 (table 1). Hepatic dysfunction was manifested by elevations in serum bilirubin, SGOT, and, occasionally, alkaline phosphatase. It was considered severe only when values were elevated to more than three times the normal value. Progressive jaundice was suspected to have contributed to the death of only one patient. In all cases but one, renal dysfunction, manifested by elevated BUN and creatinine, was related to hyperuricemia in patients who had not received allopurinol, which we now recommend for all patients receiving rubidazone as induction therapy for acute leukemia. In the other patient, acute renal failure followed hypotension and documented septicemia; thus, direct nephrotoxicity of rubidazone was not demonstrated. Toxic effects were significantly more frequent and severe at the high-dose levels.

Myelosuppression was not considered to be a toxic effect during induction therapy. During consolidation therapy at a dose of 300 mg/m^2, the median lowest recorded wbc count was 600/μl with a median of 12 granulocytes/μl and 27,000 platelets/μl. The lowest blood cell counts occurred on Day 11 (median), and recovery was complete by Day 18 (median). During maintenance therapy with a median dose of 200 mg/m^2, the time course was similar, and the median lowest wbc count was 1500/μl with a median of 420 granulocytes/μl and 62,000 platelets/μl. Despite the low blood cell counts, no patient required hospitalization for presumed infection during consolidation or maintenance therapy.

Cardiotoxicity.—Seven patients with acute leukemia in phase I–II studies had cardiotoxicity manifested by congestive heart failure (CHF), which was fatal in six of these patients (11). Cumulative rubidazone doses ranged from 1050 to 2600 mg/m^2 but all patients had had previous anthracycline therapy: 100–270 mg/m^2 of Adriamycin or 340–385 mg/m^2 of daunorubicin. An ECG was available prior to CHF in five patients and showed a QRS voltage decrease of $> 30\%$ in three and $> 25\%$ in four. Two patients underwent endomyocardial biopsy. One patient with a parotid carcinoma had received 1550 mg/m^2 of rubidazone but no prior Adriamycin and had grade 2 biopsy changes equivalent to those observed after a total Adriamycin dose of 550 mg/m^2. One of the patients with leukemia who had a biopsy after 950 mg/m^2 of rubidazone and 150 mg/m^2 of Adriamycin had similar changes. Further studies of rubidazone-treated patients with endomyocardial biopsy are planned.

Phase II Study: Acute Leukemia

Thirteen of 32 patients (42%) with AML treated at our phase II dose levels and seven of ten (70%) with ALL achieved CRs. Patients who were > 50 years old did not do as well as those patients who were < 50 years old (five of 13 [38%] versus 15 of 39 [52%]) in contrast to our findings during the phase I–II study where four of seven patients (57%) > 50 years old had CRs. Since that time, only one of six patients (17%) > 50 years old has responded. The median number of drug courses to CR is one and patients previously exposed to Adriamycin, but not known to be resistant, have responded as well as those without prior anthracycline therapy. Three of seven patients clinically resistant to Adriamycin have achieved CR with rubidazone.

Phase II Combination Study: Acute Leukemia

Fifteen of 27 previously untreated patients who were > 50 years old obtained CR with the ROAP protocol. The remission rate was higher in the 15 patients treated with the high-dose regimen (73%) than in the 12 patients who received the low-dose regimen (33%). Although this finding was perhaps dose-related, five of the patients treated with low-dose therapy had a history of preleukemia, a poor prognostic sign, compared with one of 15 treated with the high-dose regimen. Nonetheless, there was a better response rate in patients without preleukemia in the high-dose regimen (ten of 14, 71%) than in the low-dose regimen (four of seven, 57%). No unusual toxicity and no cardiotoxicity were observed.

Phase I Study: Solid Tumors

The best responses were seen in patients with carcinoma of the parotid. Two of four evaluable patients showed objective evidence of tumor regression, although the tumors were poorly measurable and it is not possible to state that true partial remission (PR) was achieved. Improvement was characterized by a resolution of thrombocytopenia and a clearing of bone marrow involvement in one patient and by a decrease in a mass within the external auditory canal in another patient. The other two patients showed stabilization of disease. Results in thyroid cancer were disappointing. In a tumor with a 40% response rate to Adriamycin, we expected to see remissions among the six patients treated. No patients had received prior therapy but none responded to rubidazone. One patient with small-cell carcinoma of the lung had a short response in liver metastases (less than a PR). Two other patients, one with adenocarcinoma of the lung and one with bladder carcinoma, had stable disease. Five patients whose disease progressed with rubidazone treatment were subsequently treated with Adriamycin. One patient with medullary thyroid carcinoma showed tumor regression (less than a PR) and another had a 2-month stabilization of previously progressive disease.

Toxicity.—In contrast to the results in acute leukemia where doses of rubidazone were substantially higher, the doses used in solid tumors were limited almost exclusively by myelosuppression. Although granulocytopenia (median lowest count, 200/μl) was overly severe in the first course in eight patients treated at a dose of 200 mg/m^2, in 14 patients the dose was well-tolerated in subsequent courses with a median lowest granulocyte count of 700/μl. Thrombocytopenia was not a frequent problem. There were five infectious episodes including one case of septicemia. Other toxic effects among 70 courses of treatment included nausea and vomiting reported in nine courses (severe in only one patient); diarrhea reported in two courses; stomatitis (severe in one course at 400 mg/m^2, severe in another course at 150 mg/m^2 in a patient with abnormal liver function, and minimal in a third course); and superficial phlebitis reported in one course. No cardiotoxicity was noted except on biopsy.

Phase II Study: Breast Carcinoma

No patient showed evidence of objective tumor regression. One patient had stable disease for 5 months. Most patients received only two to three courses and two died within 1 month of their first dose. Toxicity was mild and similar to that seen in the phase I solid tumor study.

DISCUSSION

Rubidazone is clearly an active antileukemic agent. Confirmatory studies of its single-agent activity are underway in the Southwest Oncology Group (SWOG) together with confirmatory studies of the ROAP results. In a pilot study of ROAP, 45 of 93 patients ≥ 50 years old (48%) and 15 of 16 patients < 50 years old (94%) achieved CRs (12). The newly activated phase III study will contain a direct, front-line, randomized comparison of ROAP versus Adriamycin-OAP with a modified ara-C schedule in both arms. Factors to be analyzed include CR rate, time to CR, duration of CR, survival, and toxicity.

In our preliminary studies in solid tumors, rubidazone appears less active than Adriamycin against breast and thyroid carcinomas. A study of the activity of rubidazone in breast cancer is being conducted by SWOG and a randomized comparison with Adriamycin is being conducted at the Mayo Clinic. Other Adriamycin-sensitive tumors being studied by SWOG include lymphoma, childhood leukemia, sarcomas of bone, and mesothelioma. These studies are newly activated or insufficiently advanced to report on at this time. A study in bronchogenic carcinoma and colorectal carcinoma appears discouraging, with only one response among 26 patients with colorectal carcinoma and no responses among 16 with bronchogenic carcinoma (13). The study in colorectal cancer is particularly important since rubidazone or other anthracycline analogs need not be limited in activity to Adriamycin-sensitive tumors.

Rubidazone has been claimed to be less cardiotoxic than Adriamycin in experimental animals. Certainly, its cardiac toxicity in patients with prior anthracyclines is substantial at the doses needed for its single-agent activity in acute leukemia. The finding of a grade 2 endomyocardial biopsy after 1550 mg/m^2 of rubidazone in a patient previously untreated with anthracyclines suggests that the maximum permissible cumulative dose of rubidazone may be 1500 mg/m^2, not much more than three induction doses in acute leukemia. Further studies of endomyocardial biopsy in patients not previously treated with other anthracyclines are clearly indicated and are, in fact, underway at our institution.

The future role of rubidazone will probably be limited to the treatment of acute leukemia. Like daunorubicin, it is severely myelosuppressive, which may account for its activity against acute leukemia. The present comparative studies of rubidazone and Adriamycin in combination with ara-C infusions by SWOG and the daunorubicin-Adriamycin comparison by the Cancer and Leukemia Group B will clarify the role of the various anthracycline analogs in primary combination chemotherapy for acute leukemia.

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Clinical Study of Aclacinomycin A[1]

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SUMMARY

A phase I study of a new anthracycline antibiotic, aclacinomycin A, was performed in a total of 15 patients with advanced malignancy to determine the maximum-tolerated dose. The gastrointestinal toxicity which occurred was not dose-related and was not severe. Epilation and stomatitis were extremely minimal. Both hepatic dysfunction and hematologic toxicity were dose-limiting. A recommended dose for phase II study was determined to be 2.5–3.0 mg/kg (approximately 100–120 mg/m^2) given in 3-week intervals. Objective response was observed in two patients with malignant lymphomas.

[Cancer Treat Rep 63:931–934, 1979]

In 1975, a new anthracycline antibiotic, aclacinomycin A, was isolated from a culture filtrate of *Streptomyces galilaeus*, MA144-M1 (1–3). Aclacinomycin A inhibited RNA synthesis of L1210 cells in vitro and acted on the late S phase of the cell cycle of L cells in tissue culture.

The compound has shown wide antitumor activity against ascitic and solid forms of various malignancies in animal tumor systems (2,3): L1210 and P388 leukemias, Ehrlich ascites tumor, B16 melanoma, and others. The antitumor activity of aclacinomycin A in L1210 and P388 mouse leukemias was comparable to that of Adriamycin, but at two times the dose level of Adriamycin.

The animal experiments using daunorubicin-resistant L1210 and Adriamycin-resistant P388 indicated that aclacinomycin A had cross-resistance to both agents. Animal toxicology studies (2,3) in hamsters showed fewer hematologic and cardiac toxic effects compared to those observed with daunorubicin or Adriamycin.

Furue and co-workers (4) reported the results of an initial clinical trial of aclacinomycin A in 1977. Their starting dose of 0.4 mg/kg was based on LD50 levels in animals with aclacinomycin A and Adriamycin. Gastrointestinal toxicity was suggested to be dose-limiting and clinical responses were obtained in patients with gastric cancer, lung cancer, breast cancer, and chronic myelogenous leukemia.

The second clinical study was reported by Sakano and co-workers (5) in 1978. These investigators escalated the doses from 15 to 70 mg/m^2 in 22 patients. Hematologic toxicity was found to be dose-limiting in this latter study.

In 1977, we initiated a phase I study of aclacinomycin A to determine its maximum-tolerated dose.

MATERIALS AND METHODS

A total of 15 patients were entered in the phase I study; among them, 14 had had various prior chemotherapy regimens to which they appeared to be refractory (table 1).

One patient with multiple metastases of hemangiopericytoma to the lung relapsing after surgery had had no prior chemotherapy. All patients had well-preserved bone marrow, hepatic, and renal function, but two had cardiac disease (mitral stenosis and clinically suspected cardiomyopathy due to Adriamycin).

The initial dose of aclacinomycin A was 0.6 mg/kg based upon data reported by Furue and co-workers (4). Up to 1 mg/kg, the drug was administered daily or every other day by iv push, and thereafter the administration schedule was changed to a weekly dose by drip infusion dissolved in 100–200 ml of 5% glucose in water. Later, doses ranging from 2.5 to 4 mg/kg were administered in 2–3-week intervals due to hematologic toxicity. The duration of the observation period ranged from 2 weeks to 3 months following the administration of the drug.

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TABLE 1.—Phase I study of aclacinomycin A

Patient No.	Age (yrs)	Sex	Body weight (kg)	Diagnosis	Dose (mg/day) and schedule	Total dose (mg)
1	54	F	33	Leiomyosarcoma	20 × 7 days	140
2	48	F	34	Lung (adenocarcinoma)	20 × 6 days, 30 × 2 days	180
3	71	F	35	Reticulum cell sarcoma	20 × 3 days, 30 × 3 days, 40 × 3 days	270
4	25	F	36	Lung (adenocarcinoma)	30 × 1 day, 40 × 1 wk	70
5	55	M	46.5	Gastric carcinoma	40 × 1 day	40
6	70	M	42	Rectal carcinoma	50 × 1 day, 60 × 1 day, 70 × 1 day, 80 × 1 day, 90 × 1 wk	350
7	66	F	27	Reticulum cell sarcoma	54 × 1 day	54
8	48	F	32	Breast carcinoma	64 × 1 day, 80 × 1 wk	144
9	52	F	40	Hodgkin's disease	80 × 1 day, 100 × 2 wks	280
10	55	M	65	Lung (adenocarcinoma)	130 × 1 day	130
11	44	F	45	Ovarian carcinoma	120 × 2 days, q3wks	
12	58	M	53	Hemangiopericytoma	106 × 1 day, 132.5 × 1 wk, then 159 × 1 day, 185 × 1 day at 3-wk intervals	582.5
13	70	M	50	Reticulum cell sarcoma	200 × 1 day, 150 × 1 day at 4-wk intervals	350
14	50	M	74	Leiomyosarcoma	250 × 1 day	250
15	40	M	56	Reticulum cell sarcoma	220 × 1 day	220

RESULTS

Gastrointestinal toxicity.—Mild anorexia was observed in 11 (73%) of the 15 patients and persisted for several days in some. Nausea and vomiting occurred after 2–5 hours in 11 patients and was mild to moderate; within a few hours, it subsided without the use of an anti-emetic agent. Mild diarrhea was observed in one patient. Stomatitis was not observed. Overall, gastrointestinal toxic effects had no clear-cut relation to the dose and were not severe (table 2).

Hepatic toxicity.—Abnormal elevations of SGOT and SGPT were noted in four patients 3–7 days after the administration of ≥ 3.5 mg/kg of the drug. These changes were transient and returned to the previous level within 7–11 days. Alkaline phosphatase levels were not significantly modified. Hepatic toxicity seemed to be dose-related and therefore further dose escalations were discontinued (table 3).

Hematologic toxicity.—In the 0.6–2.0-mg/kg dose range leukopenia and thrombocytopenia were minimal and normalized in a few days. In the 2.1–3.0-mg/kg dose range leukopenia reached a nadir within 4–7 days after drug administration and was corrected in about 1 or 2 weeks. Minimal thrombocytopenia developed simultaneously but disappeared in a few days. Doses of > 3.5 mg/kg induced severe leukopenia and thrombocytopenia in 7–18 days with recovery in about 2 weeks. With these doses, thrombocytopenia reached a nadir a few days earlier than leukopenia. These results indicate that hematologic toxicity was a dose-limiting factor of aclacinomycin A (table 4).

Dermatologic and other toxicity.—Minimal epilation was observed 1 week after drug administration in two patients. One patient complained of moderate vasalgia at the infusion site but neither phlebitis nor local necrosis was seen. Skin rash was observed in three patients and was accompanied by a transient mild elevation of blood pressure in one. Fever, probably drug-related, was observed in two patients but regressed spontaneously. Clinical cardiovascular signs and electrocardiographic (ECG) changes were not observed in any patient including two who had heart disease.

Several points were clarified by our study:

(*a*) Gastrointestinal toxic effects such as anorexia and nausea and vomiting were not related to dose and schedule.

(*b*) Epilation and stomatitis were extremely minimal.

(*c*) Both hepatic and hematologic toxic effects were dose-limiting.

(*d*) The recommended dose for phase II studies was 2.5–3.0 mg/kg (approximately 100–120 mg/m^2) given at 3-week intervals.

(*e*) The cardiotoxicity of aclacinomycin A relative to that of Adriamycin is still uncertain.

Table 2.—Toxicity of aclacinomycin A

Patient No.	Toxic effects*						
	Hematologic	Anorexia	Nausea	Vomiting	SGOT	SGPT	Other†
1	+	+	+	+	–	–	+
2	–	+	+	+	–	–	+
3	+	+	–	–	–	–	–
4	–	+	+	+	–	–	–
5	–	–	–	–	–	–	–
6	+	+	+	+	–	–	–
7	+	–	–	–	–	–	–
8	–	+	+	+	–	–	+
9	+	+	+	+	–	–	+
10	+	+	+	+	–	–	–
11	–	–	+	+	–	–	–
12	+	+	+	+	+	+	+
13	+	+	–	–	+	+	–
14	+	–	+	+	+	+	+
15	+	+	+	+	+	+	–

*+ = toxic effects observed; – = no toxic effects observed.

†Other toxic effects = minimal epilation in 2 patients, hypertension in 1, fever in 2, vasalgia in 1, diarrhea in 1, and flush in 3.

Table 3.—Pattern of liver toxicity

Patient No.	Single aclacinomycin A dose	Liver function test	Values (mg/100 ml) before drug dose	Values (mg/100 ml) on Day—							
				3	4	5	6	7	9	11	18
12	185 mg	SGOT	41			75			52		
	3.5 mg/kg	SGPT	61			156			96		
	123 mg/m^2	Alkaline phosphatase	56			73			79		
13	200 mg	SGOT	36					72		36	28
	4 mg/kg	SGPT	45					77		60	26
	133 mg/m^2	Alkaline phosphatase	286					261		202	876
14	250 mg	SGOT	25		306			73	58	30	
	3.5 mg/kg	SGPT	16		156			85	54	36	
	133 mg/m^2	Alkaline phosphatase	77		76			87	90	80	
15	220 mg	SGOT	26	117			47			30	
	4 mg/kg	SGPT	26	95			41			21	
	133 mg/m^2	Alkaline phosphatase	69	81			63			55	

Table 4.—Hematologic toxicity

Aclacinomycin A dose range (mg/kg)	No. of patients entered	Total No. of patients with toxicity	Toxicity occurring on Days (range)—	Nadir range (cells × 10^3/mm^3)	Recovery occurring on Days (range)—
			Leukopenia		
0.6–1.0	5	3	2–5	1.6–3.8	1–2
1.1–2.0	8	3	3–21	1.3–1.5	7
2.1–3.0	5	4	4–7	0.5–4.0	4–17
3.1–4.0	4	4	13–18	0.3–2.1	7–16
			Thrombocytopenia		
0.6–1.0	5	2	5–7	6.5–7.5	4
1.1–2.0	8	1	9	7.7	13
2.1–3.0	5	2	7–11	7.5–8.0	4
3.1–4.0	4	4	7–10	0.4–3.5	4–16

Clinical Responses

In a phase I study, investigations of clinical response are not essential; however, clinical improvement was observed in two patients with malignant lymphomas. One patient (No. 13) with reticulum cell sarcoma who had received prior treatments with radiation, VCP (vincristine, cyclophosphamide, and prednisolone), and vinblastine achieved partial remission. Another patient (No. 9) with stage IV Hodgkin's disease had a high fever related to bone marrow infiltration and became afebrile for 1 week following drug administration. All other patients showed progressive disease during the study.

DISCUSSION

The initial report by Furue and co-workers (4) suggested that gastrointestinal toxicity appeared to be a dose-limiting factor of aclacinomycin A. In this study we found that gastrointestinal toxicity was not dose-dependent. One patient (No. 12) received four administrations of the drug at escalating doses from 2 to 3.5 mg/kg weekly or in 3-week intervals (table 1). No gastrointestinal toxic effects were observed at doses of up to 3 mg/kg but anorexia and nausea and vomiting occurred at doses of 3.5 mg/kg (185 mg). Therefore, gastrointestinal toxicity might occur more frequently at higher doses.

Hepatic dysfunction was not observed at doses of up to 3.0 mg/kg but developed in four patients who received doses of 3.5–4.0 mg/kg; this was transient and disappeared within about 1 week. Furue and his co-workers (4) reported transient elevation of SGPT, LDH, and alkaline phosphatase in five of 22 patients. Although these changes were transient and reversible, the drug should be carefully administered to patients with liver dysfunction.

With respect to hematologic toxicity, a weekly administration was possible up to the daily dose of 2.0 mg/kg. When the dose was escalated to 2.5 mg/kg, 2–3-week intervals were necessary for recovery from leukopenia and thrombocytopenia. At doses of 3.5–4 mg/kg, leukopenia was severe and the nadir was reached in 2 weeks. However, recovery was relatively prompt and return to prior levels was seen in approximately 1 week. No drug-related anemia was observed.

The total dose administered ranged from 40 to 582.5 mg. No clinical signs of cardiovascular impairment or ECG changes were detected.

In a toxicologic study with hamsters (3), ECG changes such as arrhythmia and changes of T wave were reported at 50–75-mg/kg doses, but they were milder compared to those changes occurring with lower doses of Adriamycin. Thus, the cardiotoxicity of aclacinomycin A was estimated as approximately one fifteenth that of Adriamycin. Furthermore, it is of interest that these ECG changes were reversible after discontinuation of the drug.

In clinical trials, Yamagata and co-workers (6) recently reported that palpitation and sinus tachycardia were observed in three patients who received a cumulative dose of aclacinomycin A of > 1000 mg. In a summary of the present status of the clinical results by Oka (7), ECG changes such as sinus tachycardia, arrhythmia, extrasystole, and ST-T changes were reported in ten of 197 patients. These changes usually occurred a few days after the first administration and returned to normal within a few hours.

These observations suggested that the cardiotoxicity of aclacinomycin A was mild and transient and might be reversible. Comparison of the cardiotoxicity of aclacinomycin A with that of Adriamycin will be clarified with more experience at cumulative doses of aclacinomycin A of > 500 mg/m^2.

Objective response was obtained in two patients with malignant lymphomas in our study. Yokokawa and co-workers (8) reported a 1-B response in one patient with metastatic lymph nodes of primary-unknown anaplastic cell cancer. Konno et al (9) obtained transient tumor shrinkage in two of 14 patients with lung cancer (adenocarcinoma and large-cell anaplastic type). Recently, Oka (7) summarized the results of a phase I–II study involving major institutions in Japan. Objective responses were observed in patients with lymphomas, breast cancer, ovarian cancer, lung cancer, gastric cancer, and bladder carcinoma using iv and ip infusion or bladder instillation. In addition, a preliminary report by Mathé[3] indicated antitumor activity against acute leukemias. Since these encouraging results of aclacinomycin A have generated a number of phase II–III studies, the antitumor spectrum of aclacinomycin A will be ascertained in the near future.

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Anthracycline Analogs: Re-assessments and Prospects

Stephen K. Carter [1,*]

Although there are many anthracycline analogs available to us, we have no way as yet to fully utilize them all. Analogs pose strategic problems in their clinical evaluation different from those of new structures. Scattered responses to a new anthracycline analog in breast cancer or sarcomas are only minimally interesting compared to the excitement the same response would generate resulting from a new structure. Clear-cut superiority to Adriamycin must be demonstrated with any new anthracycline analog. This superiority could manifest itself as a higher response rate, a broader spectrum of activity, or diminished toxicity (ideally, cardiac toxicity). The clinical resources required to adequately test for any of the above are not insignificant. Patients available for clinical research are a valuable resource, and the funding to support such research has plateaued in recent years.

An anthracycline analog study in advanced breast cancer must compete for its strategic priority with new combination regimens, combined drug and hormone studies, and phase II studies of new structures. The success of combinations in advanced breast cancer means that for analog evaluation we must use patients who have failed to respond to prior combination regimens but have not as yet been exposed to Adriamycin. The response rate achieved in this situation must be compared to the response rate that would be achieved with Adriamycin in the same situation. The most precise tool for making this comparison is the randomized clinical trial which requires a large outlay of clinical resources. To rely on the historical control of past Adriamycin experience requires a larger therapeutic advantage for confidence as well as confirmatory repetitive studies. Since Adriamycin is active in a broad spectrum of tumors, the potential clinical trials are numerous. If an analog is clearly superior in one tumor, testing in other tumors will rapidly follow. If the first two tumors evaluated produce negative results, how many more should be tested?

Much analog development in the anthracycline area is aimed at diminishing cardiac toxicity. The clinical strategy for achieving this is more complex and less fully developed than the strategy for an increased efficacy test. The end-point in such a toxicity evaluation is not a tumor response rate but a cardiac complication rate. This end-point is less precisely established and unlike tumor response has a negative connotation for the patient's future survival. It is almost always easier to evaluate a positive end-point than a negative end-point.

Cardiac toxicity occurs chronically, and chronic toxic effects pose greater difficulties for clinical trial strategies than do acute toxic effects. To establish a meaningful cardiac toxicity potential for an anthracycline requires that patients achieve responses and tolerate maintenance therapy long enough for the cardiac toxicity to manifest itself. This requires a large number of patients at the start of a trial; in the US, a proper informed consent form for such a trial is not easy to contemplate. With many analogs in current study, data exist only for one or two courses of treatment. The lack of cardiac toxicity in this setting is again of minimal interest. Since the same would be true after one or two cycles of Adriamycin, such data give us almost no ability to compare cardiac toxicity potential.

The endomyocardial biopsy data from Stanford University, Calif, offer the exciting potential of having a pathologic end-point of cardiac toxicity available before a patient develops any clinical manifestation of the problem. Thus, it offers increased precision with diminished risk to the patient in an analog clinical study geared to evaluate cardiac toxicity potential.

Analogs of Adriamycin currently under clinical evaluation in the US include rubidazone from France, carminomycin from the Soviet Union, and AD-32 from the US. Additional drugs under clinical study throughout the world include aclacinomycin A from Japan, quelamycin from Spain, 4′-epiadriamycin from Italy, and detorubicin from France. Thus, at least seven analogs are being clinically compared with Adriamycin and daunorubicin. This is a situation fraught with both opportunity and the potential for waste. The opportunity exists to develop important data on structure-activity relationships which would correlate experimental results with clinical predictability. To accomplish this there needs to be adequate communication, coordination and collaboration of therapeutic strategies, and end-results reporting. The danger with so many analogs in clinical trials is that a massive amount of uncoordinated data will result which will not allow any meaningful decisions to be made. This will lead to competing claims, a waste of patient resources, and

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a step backward for drug development in cancer worldwide.

The major predictive systems for all analogs currently under study have been efficacy systems, predominantly rodent transplantable tumors. The tumor systems used have been those which initially predicted the clinical activity of Adriamycin, such as Ehrlich ascites carcinoma, L1210 and P388 leukemias, and B16 melanoma. It is not known how predictive these systems will be for analog superiority or the lack thereof. As with new drug screening, only the positive drugs receive clinical testing and therefore the false-negative rate can never be truly determined. Since the clinical evaluation of an analog is more complex than that of a new structure, the determination of the clinical predictiveness of experimental systems is that much more difficult. It is worth noting that many of the analogs discussed in other papers in this issue have not shown clear-cut higher activity than Adriamycin in the major predictive systems. A panel approach using many varied systems is intellectually more attractive for analogs than pinning all hopes on a single system such as L1210 or P388 leukemia. More coordinated thought needs to be given to the design of analog prediction panels and the therapeutic strategy for testing their applicability in man.

Cardiac toxicity animal models are still in their infancy when compared to the efficacy models. Some models are acute models such as the rat ECG model of Zbinden. Some models are chronic such as the rabbit system which is the most popular at this time. The acute systems have the advantage of relative low cost with the resultant ability to test larger numbers of compounds. What is not known is how relevant the acute models are for the chronic cardiomyopathy, which is the clinical problem. The rabbit model conceptually is closer to the human toxicity, but is both expensive and time-consuming, which does not make it feasible to screen large numbers of compounds. An additional difficulty is that until now all analogs tested have shown quantitative rather than qualitative differences in comparison with Adriamycin. No compound yet tested has shown evidence of antitumor effect in experimental systems and has been totally devoid of cardiac toxicity in the rat or rabbit models. Quantitative differences between an analog and Adriamycin must be interpreted cautiously unless it can be demonstrated that the clinically effective dose level of the analog will be comparable to that of Adriamycin. What should be explored are ratios of LD10 doses or maximally effective doses in the rodent systems and their correlation with the levels which cause cardiac toxicity in the rat or rabbit. An important beginning could be made with the numerous analogs in the clinic so that correlations with the human maximum tolerated dose and common clinical dose levels could be made. This would be an important task for future consideration.

ANNOUNCEMENTS

American Board of Internal Medicine Examinations

The next examinations in Hematology, Infectious Disease, Nephrology, Pulmonary Disease, and Rheumatology will be on June 17, 1980. Registration for these subspecialty examinations will begin August 1, 1979, and will continue through November 1, 1979. For those who began their residency training in internal medicine before June 1, 1970, and who have < 2 years of acceptable subspecialty training this will be the last opportunity for admission to a subspecialty examination. *Applications may be obtained from:* The American Board of Internal Medicine, University City Science Center, 3624 Market St, Philadelphia, Pa 19104 (telephone: 215-243-1500).

The American Board of Internal Medicine has announced a significant change in policy regarding its training requirements. This change will affect physicians desiring admission to a subspecialty examination who began their residency training in *internal medicine* before June 1, 1970. At present, these physicians are considered to have met the training requirements for admission to a subspecialty examination after completing 1 year of acceptable training in the subspecialty. If once informed of admission to a subspecialty examination, these physicians *will continue* to be considered to have fulfilled the Board's training requirements in that subspecialty. Beginning with the subspecialty examination offered in 1981, however, *all physicians* not previously admitted to an examination will be required to meet the same training requirements which apply to physicians beginning their residency training after June 1, 1970. These requirements currently involve 2 full years of acceptable training in the subspecialty. A complete description of the Board's policies and procedures may be obtained by directing a written request to the American Board of Internal Medicine (above address).

Course on "Contemporary Cancer—the Experts' View"

Kona, Hawaii, August 25–September 1, 1979

The University of Hawaii Cancer Center announces a course on "Contemporary Cancer—the Experts' View" to be held at the Mauna Kea Beach Hotel in Kona, Hawaii, from August 25 to September 1, 1979. This is a limited-registration course providing an in-depth but leisurely conceptual overview of cancer which should be of interest to oncologists in all disciplines. It qualifies for 29 hours of CME credit. The faculty include Drs. Jerome J. deCosse, Charles Heidelberger, James F. Holland, Jimmie Holland, and Henry S. Kaplan. *For registration or further information write to:* Dr. Larry Hagmann, University of Hawaii at Manoa, College of Continuing Education and Community Service, 2530 Dole St, Rm C–104, Honolulu, Hawaii 96822, or phone (808) 948-8581.

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